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Production of Active Compounds against *Trichophyton rubrum* by *Bacillus* species

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Rhizospheric soil is rich of bacteria that produce a wide range of bioactive substances with antimicrobial and antifungal properties, among them bacillus genus have the ability to produce hundreds of biologically active compound that effective against microorganisms. Genus of Bacillus capable of producing biologically active compound is effective against microorganisms and appears to be potential candidates in the biocontrol of fungal pathogens. In this study, soil samples were collected from different sites in the Gorgan region in order to *Bacillus* isolation and determination their antifungal activity against *T.rubrum*. In order to isolation of these bacteria soil samples was suspended in sterile distilled water or normal saline and was serially diluted , suspensions cultured on microbiological media and then identified by biochemical methods. Isolates that had highest antifungal effects analysed with PCR and 16s rRNA sequencing. Among 54 strains, only 12 strains showed antagonistic activity against *T.rubrum*. Sr4 and Sr12 isolates showed the highest antagonistic activity and based on biochemical tests and PCR were identified as *B.cereus* and *B.thuringiensis* respectively. These isolates based on 16s rRNA sequence analysis showed 97% homology with *Bacillus cereus* strain *KU4* and *Bacillus thuringiensis* strain *ucsc27*. According to the results seems the soil Bacillus have appropriate biocontrol potential against dermatophytic agents such *T.rubrum*.

Key words: Active compounds, *Trichophyton rubrum, Bacillus* species.

Soil is the major repository of microorganisms that produce antibiotics capable of inhibiting the growth of other microorganisms. Clinically useful antibiotics have been isolated from of soil bacteria such as Bacillus sp. The continuous appearance of resistant and multiresistant pathogenic bacteria and fungi has promoted a continuous search for new antibacterial and /or antifungal drugs (Mahrath, 2009).

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The genus *Bacillus* is a major group of soil bacteria and one of the most utilized in the biocontrol of pathogens. These bacteria consist of a heterogeneous group of Gram-positive, aerobic or facultative anaerobic that have ability of producing peptide antibiotics. This Compounds contribute to the utilization of this genus on the biocontrol (Backman *et al*., 1997).

Fungal diseases can have major problems in man health and, in conventional medicine, chemical antifungal are routinely used to provide disease control. However, as these chemicals are often toxic and harmful to man, alternative methods for control are needed. Biological control is a best potential alternative approach to chemical treatment (Cornea *et al*., 2003; Mateescu *et al*.,

2003). Dermatophytosis, mycotic infections caused by dermatophytes, are commonly related in tropical countries and represent an important public health problem yet unresolved (Chineli *et al*., 2003; Weitzman *et al*., 2005; Graser *et al*., 2008) *Trychophyton rubrum*, is one of the most common species isolated in many parts of the world as causative agents of dermatophytosis(Araujo *et al*.,2009; Howard *et al*., 2002)

MATERIALS AND METHODS

Sample collection

Twenty samples of agricultural soil were collected from different localities at gorgan city. Soil samples were collected by scraping off surface material with a sterile spatula and then obtaining approximately 100-g samples from 2-5 cm below the surface, around the plants roots. Samples were then stored at 4 °C until use (Gebreel *et al*., 2008; Craford *et al*., 1993).

Isolation and Characterization

Bacteria were isolated by serial dilution and streak plate methods. The aliquots (0.1 ml) were plated in triplicates on Nutrient Agar (NA) medium [(w/v) 0.5% peptone; 0.3% beef extract; 0.5% NaCl; 1.5% agar, pH 7] and incubated at 30±2°C for 72 h (Kumar *et al*., 2009). Phenotypic characterization of the isolate was done by different tests referring to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994; Gordon *et al*., 1973).

In vitro antifungal activity

In vitro antagonistic examination, the antifungal activity of bacteria were tested against *T.rubrum* with agar well diffusion methods on the PDA media. In this method spore suspensions of fungus (five-day-old cultures with a concentration of more than 10^6 cfu/mL) were prepared in 0.85% sterilized saline then 2ml spores suspension of test organism was spread on the agar surface of the plate, then two equally spaced wells of 6mm diameter were made in the agar. All plates then incubated for 48-96 h in 37°C and inhibition zone diameter was measured in mm(El-Mehlavi *et al*., 2008; Gebreel *et al*., 2008).

Molecular characterization

In order to characterization of *Bacillus* isolates by genotypic characterization and molecular methods, PCR and 16S rRNA sequencing

Table1. Biochemical characterization of antagonistic *Bacillus* isolates

No	Catalase	Glucose	VP	Citrate	Nitrate	Lipase	Gelatin	Starch	Motility	Casein	Parabasal body
Sr1	+	+	+	+	+	+	+	+	+	+	-
Sr2	+	+	+	+	+	+	+	+	+	+	-
Sr3	+	+	+	+	+	-	+	+	+	+	-
Sr4	+	+	+	+	+	-	+	+	+	+	-
Sr5	+	+	+	+	+	-	+	+	+	+	-
Sr6	+	+	+	+	+	+	+	+	+	+	-
Sr7	+	+	+	+	+	+	+	+	+	+	-
Sr8	+	+	+	+	+	+	+	+	+	+	-
Sr9	+	+	+	+	+	+	+	+	+	+	-
Sr10	+	+	+	+	+	+	+	+	+	+	-
Sr11	+	+	+	+	+	+	+	+	+	+	-
Sr12	+	+	+	+	+	+	+	+	+	+	-

Table 2. Antifungal activity of ten isolates of *Bacillus* sp. against *T.rubrum.* (3 readings)

Isolate	Inhibition zone diam(mm)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	22	23	21	23	22	22	18	20	20	21	22	22
2	26	18	23	25	20	23	24	24	22	18	23	23
3	22	21	22	24	21	20	20	22	22	19	23	20
Mean	23.6	20.6	22	24	22	21.6	20.6	22	21.3	19.3	22.6	21.6

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>gb|JF895480.1| Bacillus cereus strain KU4 16S ribosomal RNA gene, partial
sequence
Length=1453
 Score = 1624 bits (879), Expect = 0.0
 Identities = 950/983 (97%), Gaps = 9/983 (1%)
 Strand=Plus/Plus
Query 1    GAGCGAATGGATTGAGAGCTTGCTCTCAAGAAGTTAGCGGCGGACGGGTGAGTAACACGT  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 30   GAGCGAATGGATTGAGAGCTTGCTCTCAAGAAGTTAGCGGCGGACGGGTGAGTAACACGT  89
Query 61   GGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACA  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 90   GGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACA  149
Query 121  TTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCC  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 150  TTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCC  209
Query 181  GCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACC  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 210  GCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACC  269
Query 241  TGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC  300
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 270  TGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC  329
Query 301  AGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAA  360
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 330  AGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAA  389
Query 361  GGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGG  420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 390  GGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGG  449
Query 421  CACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT  480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 450  CACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT  509
Query 481  ACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTT  540
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 510  ACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTT  569
Query 541  AAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTG  600
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 570  AAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTG  629
Query 601  AGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGG  660
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 630  AGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGG  689
Query 661  AACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGG  720
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 690  AACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGG  749
Query 721  GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA-CGATGAGTGCTAAGTGTT  779
           |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct 750  GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT  809
Query 780  AGAGGGTTTCCACCCTTTAGTGCTGAAGTTAACGCACTAAGCACTCCGCCTGGGGGAGTA  839
           |||||||||||| |||||||||||||||||| |||||||||||||||| ||||
Sbjct 810  AGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGG-AGTA  868
Query 840  CGGCCGCGACGCTGAGGCTCAAAGGAATTGACTGGGGCGCGGCACA-GCTGTGGAGCATG  898
           ||||||| | |||||  ||||||||||||||| ||||| || |||| || ||||||||||
Sbjct 869  CGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG-CACAAGCGGTGGAGCATG  927
Query 899  TGGTTTGTATTTTTAGCAACAACGAAGAAGCTTACCAGGTCT-GAGTTC-TGCTGACAAC  956
           ||||||  |||   ||||||  ||||||| |||||||||||  ||  || | |||| |||
Sbjct 928  TGGTTTA-ATTCGAAGCAACG-CGAAGAACCTTACCAGGTCTTGACATCCT-CTGAAAAC  984
Query 957  CGGAGAGCTTTGGCTTCTCGTTC  979
           |  |||| |  |||||||| |||
Sbjct 985  CCTAGAGATAGGGCTTCTCCTTC  1007
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Fig. 1 .97% homology of sr4 isolate(Rev primer) with Bacillus cereus strain KU4

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>emb|FN667913.1| Bacillus thuringiensis partial 16S rRNA gene, strain ucsc27
Length=1355
Score = 2039 bits (1104), Expect = 0.0
 Identities = 1202/1243 (97%), Gaps = 33/1243 (3%)
 Strand=Plus/Plus

Query 2    AGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAAC  61
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 5    AGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAAC  64
Query 62   ACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGAT  121
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 65   ACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGAT  124
Query 122  AACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGG  181
           || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 125  AAYATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGG  184
Query 182  ACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCC  241
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 185  ACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCC  244
Query 242  GACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG  301
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 245  GACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG  304
Query 302  CAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGA  361
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 305  CAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGA  364
Query 362  TGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAG  421
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 365  TGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAG  424
Query 422  CTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG  481
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 425  CTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG  484
Query 482  TAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT  541
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 485  TAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT  544
Query 542  TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGA  601
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 545  TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGA  604
Query 602  CTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATG  661
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 605  CTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATG  664
Query 662  GAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGC  721
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 665  GAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGC  724
Query 722  GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAG  781
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 725  GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAG  784
Query 782  TGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGA  841
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 785  TGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGA  844
Query 842  GTACGGCCGCAAGGCTGAAACTCAA-GGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  900
           |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct 845  GTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  904
Query 901  TGTGGTTTAATTCGAAGCAACGCGA-GAACCTTACCAGGTCTTGACATCCTCTGAAA-CC  958
           ||||||||||||||||||||||||| |||||||||||||||||||||||||||||||| ||
Sbjct 905  TGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACC  964
Query 959  CTAGAGATAGGGCTTCTCCTTCGG-AGCAGAGTGACAG-TG-TGCATG-TTGTCGTCAGC  1017
           |||||||||||||||||||||||| ||||||||||||| || |||||| ||||||||||||
Sbjct 965  CTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGC  1022
Query 1015 TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA-CGAGCGCA-CCCTTGATCT-AGT-GC-  1072
           |||||||||||||||||||||||||||||||| |||||||| |||||||||| ||| ||
Sbjct 1025 TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC  1082
Query 1070 ATCAT-A-GTTGGGCACTCTAAG-TGACTGCCG-TGACA-CCGGAG-A-G-TGGGGATGA  1122
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Fig. 2. 97% homology of sr12 isolate(Rev primer) with *Bacillus thuringiensis* strain ucsc27

were carried out. Genomic DNA of pure subcultures of antagonistic isolates was extracted using Sinagene Microbial DNA isolation kit , Cat.No.DN8115C (Sinagene.Iran) according to the manufacturers specifications. 45 microliter of PCR product with forward primer send to macrogene company of South Korea (www.macrogen.com) for 16S rRNA sequencing. Universal 16S rRNA primers were used (forward primer was GAGTTTGATCCTGGCTCAG and revers primer was GACGGGCGGTGTGTACAA). Sequence data was analyzed with Chromas software version 2.33. The 16s rRNA gene sequence were compared to sequence in the public database using basic local alignment search tool (BLAST) on the national center for biotechnology information (NCBI) website (www.ncbi.nlm.nih.gov). Homology of the 16s rRNA sequence of isolate was analyzed by using BLAST program (Rintala *et al*., 2001; Singh *et al*., 2009).

Statistical Analysis

All experiments were performed in a completely randomized design. The results were subjected to analysis of variance (ANOVA) and means were compared by Duncan Test ($P < 0,05$) using the software SPSS

RESULTS AND DISCUSSION

Morphological studies of isolates revealed that all 12 isolates from rhizosphere soils were gram positive bacilli with large and flat surface colony. All isolates identified using biochemical testes such as catalase, glucose, nitrate reduction, lipase production, casein, starch and gelatine hydrolysis according to Bergy's manual of systematic bacteriology (Table 1).

All of 12 *Bacillus* isolates were screened by well diffusion methods and among them 5 isolates showed antagonistic activity against *T.rubrum*. the dual culture revealed that isolates inhibited the growth of *T.rubrum* by well developed inhibition zone (Table 2).

In the tests on PDA plates, different isolates exhibited different inhibitory effects on T.rubrum. In the assay of inhibitory growth, the rhizospheric bacilli significantly suppressed the growth of *T.rubrum* ($p<0.05$). As shown in Table 2, isolates no 4 and 12 were the most effective on control of *T.rubrum* .Among the tested 12 Bacillus spp., 5 isolates showed growth inhibition against *T.rubrum* with the production of clear zones at 24 hours incubation (Table 2). The result produced by isolate no 4 was significantly ($P < 0.05$) higher than that produced by other 4 bacteria.

Two isolates, Sr4 and Sr12 had a highest antidermatophytic effects and based on biochemical testes identified as *B.cereus* and *B.thuringiensis* respectively. Blast analysis of partial 16S rRNA gene sequences showing that the Sr4 and Sr12 isolates were closely affiliated with genus *Bacillus*. These isolates shared sequence identity of 97% with *B.cereus* KU4 and *B.thuringiensis* respectively. (Figure1&2).

CONCLUSION

Antagonism is ubiquitous in nature among different species. The production of antifungal activities occur during growth of *Bacillus thuringiensis* and *B.cereus* on solid media that are the most common saprophytic bacteria exist in the soil and are known for their potential as antibiotic producer. The result of the present investigation reveal that the *Bacillus thuringiensis* and Bacillus thuringiensis strain ucsc27 isolated from soil show potential antidermatophytic activity against the dermatophyte *T.rubrum*. These isolates might be good candidates to dermatophytic fungi biocontrol. Further work will be undertaken to isolate and identify the compounds produced. The results of this study demonstrated for the first time that the *Bacillus thuringiensis* and *B.cereus* have great potential in controlling dermatophytic fungi such as *T.rubrum*. This finding suggested that these bacteria could inhibit the pathogens due to some toxic compounds accumulated in the culture medium or antibiotic production. This result was in agreement with that reported from other antagonists such as Bacillus sp on fungi. In conclusion, our results showed that these bacteria have potential biocontrol activity against the dermatophytes.

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REFERENCES

1. Araujo C R, Mianda K C, Fernandes O F L, Soares A J, Silva M R. In vitro Suceptibility testing of dermatophytes isolated in goiania, brazil, against five antifungal agents by broth microdilution method. *Rev. Inst. Med. trop. S. Paulo.*,2009; **51**(1):9-12.
2. Backman, P. A.; Wilson, M.; Murphy, J. F. Bacteria for biological control of plant diseases. In: N. A. Rechcigl & J. E. Rechcigl (Eds.) Environmentally Safe Approaches to Crop Disease Control, p.,1997; 95-109.
3. Chinelli P V A, Sofiatti A A, Nunes R S, Martins J E C. Dermatophyte agents in the city of São Paulo, from 1992 to 2002. *Rev. Inst. Med. trop. S. Paulo.*, 2003; **45**: 259-263.
4. Cornea C P, Mateescu R, Grebenisan I, Campeanu G. *Roum.Biotechnol.Lett.*, 2003; **8**(1) 1115 – 1122.
5. Crawford DL, Lynch JM, Whipps JM, Ousley MA. Isolation and characterization of actinomycete antagonists of a fungal root pathogent. *Applied and environmental Microbiology.*, 1993; 3899-3905.
6. El-Mehalawy AA, Gebreel HM, Rifaat HM, El-Kholy IM, Humid AA. Effect of antifungal compounds produced by certain bacteria on physiological activities of human- and plant-pathogenic fungi. *Journal of Applied Sciences Research*.,2008; **4**(4): 425-432.
7. Gebreel HM, El-Mehalawy AA, El-Kholy IM, Rifaat HM , Humid AA. Antimicrobial activities of certain bacteria isolated from egyptian soil against pathogenic fungi. *Research Journal of Agriculture and Biological Sciences.*, 2008; **4**(4): 331-339.
8. Gordon R E, Haynes W C, Pang C H. "The genus *Bacillus*," in *Agriculture Handbook*, Washington, D. C: US Dept. of Agriculture., 1973; **427**: 1-99.
9. Graser, Y., Scott, J., Summerbell, R. The new species concept in dermatophytes-a polyphasic approach. *Mycopathologia*., 2008; DOI 10. 1007.
10. Holt, J .G and et . al.1993.Bergey's manual of determinative bacteriology . Williams & wilkins., 1993; 667-675.
11. Howard D H, Weitzman I , Padhye A A. Onygenales: Arthrodermataceae in Pathogenic fungi in humans and animals/ second ed./Marcel Dekker, inc., 2002.
12. Kumar A, Pragati S, Shrivastava JN..Production of peptide antifungal antibiotic and biocotrol activity of *Bacillus subtilis. Ind J of Exprim Biol.*, 2009; **47**: 57-62.
13. Mahrath A J. The Antimicrobial Activity (Antibacterial, Antifungual) Of SomeNew 3-Substituted Isobenzofuran-1(3H)-One Derivatives. *National Journal of Chemistry*. 2009; **34**: 303-308
14. Mateescu R , Cornea C P , Grebenisan I . Dragos S, Sirean O, Jurcoane S T, Campeanu G. *Roum. Biotechnol. Lett.*, 2003; **8**(4): 1367-1374.
15. Rintala, H., Nevalainen, A ., Ronaka,A., Suutari, M. PCR primers targeting the 16s rRNA gene for the specific detection of streptomycetes. *Molecular and Cellular Probes.*, 2001; **15**: 337-347
16. Singh, V.,Praveen, V,. Khan, F and Tripathi, C. K. M. Phylogenetics of an antibiotic producing *Streptomyces* strain isolated from soil. *Biomed. Info.*, 2009; **4**(2): 53-58.
17. Weitzman, I., Summerbell, R.C. The Dermatophytes. *Clin. Microbiol. Rev.*, 1995; **8**: 240-259.