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

Hamid Reza Pordeli, Seyed Jamal Hashemi Hazaveh*, Mahmood Jamshidian and Mansour Bayat

Department of Pathobiology, College of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.

(Received: 18 February 2014; accepted: 21 April 2014)

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 uscsc27. 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 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).

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*., 1997).
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). *Trichophyton 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 10⁶ cfu/mL) were prepared in 0.85% sterilized saline then 2 ml 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.

<table>
<thead>
<tr>
<th>No</th>
<th>Catalase</th>
<th>Glucose</th>
<th>VP</th>
<th>Citrate</th>
<th>Nitrate</th>
<th>Lipase</th>
<th>Gelatin</th>
<th>Starch</th>
<th>Motility</th>
<th>Casein</th>
<th>Parabasal body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sr12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Table 1. Biochemical characterization of antagonistic Bacillus isolates**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Inhibition zone diam(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>Mean</td>
<td>23.6</td>
</tr>
</tbody>
</table>

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

Fig. 1. 97% homology of sr4 isolate (Rev primer) with Bacillus cereus strain KU4
Pordeili et al.: Study of Trichophyton rubrum by Bacillus species

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 macrogen 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).

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 tests such as catalase, glucose, nitrate reduction, lipase production, casein, starch and gelatine hydrolysis according to Beriy’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 ucs27 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.

ACKNOWLEDGMENTS

This work was supported by Tehran Science and Research Branch, Islamic Azad University. I wish to express my thanks to my supervisor Seyed Jamal Hashemi Hazaveh and
other professors in department of mycology especially Mansour Bayat and Mahmoud Jamshidian for their warms encouragement.

REFERENCES


