Removal of Phenol Using Spore Forming *Bacillus* ABO11 Isolated from Waste Water Treatment Plant

Majdah Mohamed Ahmed Aburas

Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, KSA
Email: velvetmsfs@gmail.com


Received: August 19, 2016
Accepted: October 15, 2016
Published: October 18, 2016

Copyright © 2016 by author and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution-NonCommercial International License (CC BY-NC 4.0).
http://creativecommons.org/licenses/by-nc/4.0/

Abstract

Twenty isolates of bacteria were isolated from contaminated wastewater collected from wastewater treatment station, used to clean the raw sewage at Jeddah on minimal medium containing phenol (0.5 g/l) as carbon source while at 0.6 g/l phenol, five isolates belonging to Gram positive and Gram negative bacteria were obtained after 10 days of growth at 37˚C. All the tested bacteria were tested for phenol degradation in liquid medium and isolate ABO11 was the most active isolate in phenol degradation. It was selected and tested for the ability to use phenol as carbon and energy source. It was identified as *Bacillus stercothermophilus* ABO11 using morphological and biochemical tests. Genetic studies confirmed the identification. Bacterial growth and phenol degradation by the selected bacterium were determined using different initial phenol concentrations (0 - 1.0 g/l). Very weak growth was recorded at 0.8 g/l and no growth or degradation was observed at higher phenol concentration. The isolate was adapted to grow in presence of 0.8 g/l phenol and phenol degradation was checked up to 12 days of growth. Percentage of degradation was 100% after 10 days. Maximum growth was observed at 40˚C, pH 8 and using NH4Cl as nitrogen source. In conclusion, the selected isolate, *Bacillus stercothermophilus* ABO11 can be used in protecting the environment from phenol pollution.

Keywords

Phenol, Degradation, Bacteria, Wastewater, Assay, Bacillus

1. Introduction

Phenolic compounds contained a hydroxyl group (OH) directly attached to aromatic cycle and phenol (C6H5OH) is the simplest compounds in the environment that are
widely distributed as pollutants [1] [2]. Each year, millions of tones of phenol are produced for the production of plastics and caprolactam, used for nylon and synthetic fibers [3]. Phenolic compounds are detected in ground water, waste water and in effluents of much industrial waste water [4]. Nowadays, degradation of phenol is of great importance due to their toxicity and high solubility. These compounds are contaminants in environmental matrices, food, and medicine products that can easily be absorbed through humans and animals’ skin or mucous membranes. Phenols significantly inhibit microbial activity due to membrane damage at higher phenol concentrations [5] [6] and toxicity to biological treatment systems and bacterial cell disruption were observed in high phenol concentrations. In the environment, phenol can be aerobically degraded to catechol using a single microbe or a combination of microorganisms like Trichosporon cutaneum [7], Brevibacterium fuscum [8], Hormodendrum bergeri, Fusarium oxysporum and Aspergillus flavus var. coulmmaris [9]. The aims of this investigation were to isolate, characterize and identify a bacterial isolate with potential activity for phenol degradation from waste water effluents.

2. Materials and Methods

2.1. Bacterial Isolation

Row waste water samples were collected from different Wastewater Station at Jeddah and bacteria was isolated on minimal salt medium containing 0.5 g/l phenol. The minimal salt medium was composed of (g/l): NH₄Cl, 2.1; K₂HPO₄, 4.35; KH₂PO₄, 1.7; MgSO₄·7H₂O 0.2; CaCl₂·2H₂O 0.03; MnSO₄, 0.05 and CaCl₂·H₂O, 0.03. Agar, 20 g, were added for solid medium preparation. All the previous material was added to 1000 ml double distilled water and 0.5 g/l of phenol was added. Agar plates were inoculated with 0.1 ml of the waste water and all plated were incubated at 37˚C for 7 days. The growth, cell shapes and Gram reaction of all obtained isolated were detected. All the isolates were selected and purified on nutrient agar plates and pure isolates were stored on LB agar slant at 37˚C until further use.

2.2. Growth in Phenol Containing Broth Medium

All the obtained isolated were grown in nutrient broth medium for 2 days at 37˚C (preculture). All the isolates were screened in 250 ml flask containing 50 ml of minimal salt broth medium containing phenol (0.8 g/l) as sole source of carbon and with 2 ml of the preculture of the tested bacterium (2 × 10⁶ cfu/ml). All inoculated flasks were incubated at 37˚C on rotary shaking incubator at 120 rpm for 7 days [10]. Finally, growth of cells was determined by turbidity measurement at 600 nm and % of phenol degradation was determined after 7 days. Medium containing all components without bacterial inoculums was used as control.

2.3. Selection and Adaptation of the Best Phenol Degraded Isolate

Isolate ABO11 was selected for adaptation for phenol degradation in 250 ml flask containing 50 ml of minimal salt broth medium, contained 0.1 g/l and 5 ml of enriched
media was transferred to 45 ml of new prepared medium, containing subsequently increased content of phenol (0.1 - 1 g/l), each 5 days. All flasks were incubated at 37˚C at 120 rpm. The growth was detected by spreading 0.1 ml of the culture on NA medium.

2.4. Bacterial Growth in Liquid Medium

Isolate ABO11 was grown in 250 ml flask containing 48 ml of minimal salt broth medium, contained 0.8 g/l phenol and inoculated with 2 ml of the preculture (2 × 10⁶ cfu/ml) of the bacterial isolate. All flasks were incubated at 37˚C at 120 rpm and the growth (A 600 nm) and phenol concentration were determined and compared with control (uninoculated broth medium). % of phenol degradation was calculated.

2.5. Best Conditions for Phenol Degradation

Effect of different temperatures (20˚C - 50˚C) and pH values on bacterial growth were determined in minimal salt broth medium after 10 days of growth at 120 rpm. Effect of different nitrogen source were determined in the previous medium in which nitrogen source was replaced with Peptone, yeast extract, NaNO₃, and valine at concentration of 2 g/l. The used positive control was NH₄Cl. After 10 days at 40˚C, bacterial growth was determined by the absorbance at 600 nm.

2.6. Identification of the Best Phenol Degraded Isolate

The selected bacterium ABO11 was streaked on nutrient agar plates, incubated at 37˚C and examined for colony shape, Gram reaction and cell morphology using light and electron microscopes. Some biochemical tests including esculin, Tween 80, lecithin and starch hydrolysis, utilization of casein and gelatin liquefaction were determined. Identification was carried out according to Bergey’s manual of determinative of bacteriology [11] and Sivaraj et al. [12]. Molecular studies and phylogenetic analysis of 16S rDNA sequence of the isolate ABO11 was carried out as described by Tork et al. [13] and Aly et al. [14]. ABO11 genomic DNA was isolated using QIAamp DNA Mini Kit and the 16S rDNA gene was amplified by polymerase chain reaction technique [15]. The forward primer 5’ AGTTTGATCATGGTCAG-3’ and reverse primer 5’-GGTTACCTTGTTACGACT 3’ were used and the purified PCR products was sequenced using big dye terminator cycle sequence kit, analyzed using DNA sequence ABI PRISM 310 genetic analyzer (Perkin Elmer, USA) and compared to the GeneBank [13].

2.7. Phenol Assay

The residual amount of phenolic compounds present in the sample at different incubation period was measured by colorimetric assay 4-amino antipyrine method [16]-[18].

3. Results

This study was focused on isolation and evaluation of the capability of bacteria, isolated from heavily polluted water, for phenol degradation. Raw wastewater samples were collected from wastewater treatment station, used to clean the raw sewage, for bacterial
isolation on minimal salt agar medium, containing phenol (0.5 g/l) as carbon source. Twenty different bacterial isolates were obtained and screened on minimal salt agar medium, containing phenol (0.6 g/l). Five isolates belonging to Gram positive and Gram negative bacteria were obtained after 10 days of growth at 37°C (Table 1). All the tested bacteria were tested for phenol degradation in liquid minimal salt medium. The bacterial growth (A600 nm) in phenol containing medium was ranged from 0.22 - 0.52 and percentage of phenol degradation was ranged from 46% - 100%. The isolate ABO11 was the most active isolate in phenol degradation where no phenol concentration was recorded on the medium while isolate ABO1 showed the highest growth. The most active isolate ABO11 in phenol degradation was selected and tested for the ability to use phenol as carbon and energy source at higher concentrations. It was identified as *Bacillus steraseothermophilus* ABO11 using morphological and biochemical tests (Table 2). It was Gram positive, spore forming bacilli, has creamy color and regular colonies. It has no capsule or cyst. Hydrolysis of lecithin, tween 80 and esculin were negative while starch hydrolysis was positive (Table 2). Utilization of casein was positive while gelatin liquefaction was negative. Moreover, resistance to Ampicillin was negative. Genetic studies confirmed the identification (Figure 1). Bacterial growth and phenol degradation by the selected bacterium was determined using different initial phenol concentration (0 - 1.0 g/l). Very weak growth was recorded at 0.8 g/l and no growth or degradation was observed at higher concentration (Figure 2). The isolate was adapted to grow

**Table 1.** Growth and phenol degradation of the isolated bacteria from waste water on solid and in broth media.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Source of isolation</th>
<th>Solid medium</th>
<th>Liquid medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cell shape</td>
<td>Gram reaction</td>
</tr>
<tr>
<td>ABO1</td>
<td>Waste water</td>
<td>Bacilli</td>
<td>Negative</td>
</tr>
<tr>
<td>ABO7</td>
<td>Waste water</td>
<td>Cocci</td>
<td>Negative</td>
</tr>
<tr>
<td>ABO9</td>
<td>Waste water</td>
<td>Cocci</td>
<td>Positive</td>
</tr>
<tr>
<td>QBO10</td>
<td>Waste water</td>
<td>Bacilli</td>
<td>Negative</td>
</tr>
<tr>
<td>ABO11</td>
<td>Waste water</td>
<td>Bacilli</td>
<td>Positive</td>
</tr>
</tbody>
</table>


**Table 2.** The morphological and biochemical characters of the isolate ABO11.

<table>
<thead>
<tr>
<th>Character</th>
<th>Result</th>
<th>Character</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>Positive</td>
<td>Gelatin liquefaction</td>
<td>–</td>
</tr>
<tr>
<td>Acid fast</td>
<td>Negative</td>
<td>Hydrolysis of esculin</td>
<td>–</td>
</tr>
<tr>
<td>Shape</td>
<td>Bacilli</td>
<td>Hydrolysis of Tween 80</td>
<td>–</td>
</tr>
<tr>
<td>Color</td>
<td>Creamy</td>
<td>Egg yolk lecithin</td>
<td>–</td>
</tr>
<tr>
<td>Colonies</td>
<td>Regular</td>
<td>Hydrolysis of starch</td>
<td>+</td>
</tr>
<tr>
<td>Colony edge</td>
<td>Entire</td>
<td>Hemolysis</td>
<td>–</td>
</tr>
<tr>
<td>Endospore</td>
<td>+</td>
<td>Utilization of casein</td>
<td>+</td>
</tr>
<tr>
<td>Cyst, capsule</td>
<td>–</td>
<td>Resistance to Ampicillin</td>
<td>–</td>
</tr>
</tbody>
</table>
Figure 1. Phylogenetic tree based on 16S rDNA sequence comparisons of *Bacillus sterothermophilus* ABO11, using neighbor joining tree method, maximum sequence difference = 0.002.

![Phylogenetic tree](image)

**Figure 2.** Effect of different concentration of phenol on bacterial growth of the isolate ABO11.

in presence of 0.8 g/l phenol and phenol degradation was checked up to 12 days of growth (**Figure 3** and **Figure 4**). Increasing time, increased growth and phenol degradation and percentage of degradation was 100% after 10 days and negligible decrease was observed in phenol concentration in control sample. Maximum growth of the selected isolate, *Bacillus sterothermophilus* ABO11 in minimal salt broth medium with 0.8 g/l phenol as carbon source was observed at 40°C, pH 8 and using NH₄Cl as nitrogen source (**Figures 5-7**).

4. **Discussion**

Bacteria have a major role in saving the environment by removing of toxic chemicals
Figure 3. Growth of the isolate ABO11 and phenol degradation after 12 days at 37°C, 120 rpm in minimal medium containing 0.8 g/l phenol as a carbon source.

Figure 4. Phenol concentration recorded after the growth of the selected isolate ABO11 in medium containing phenol after 12 days and compared to control (non-inoculated medium).
Figures 5 and 6. Growth of the selected isolate ABO11 in medium containing phenol for 10 days at different temperature and pH values.

and wastes that are harmful to human and animals. Many authors reported that bacteria or fungi can be effectively used for bioremediation of phenol in contaminated sites [9] [19]-[21]. Degradation intermediate compounds were also determined. Isolation of bacterial that degrade chemical compounds usually carried out from polluted water or soil. Phenol can cause serious problems in the wastewater treatment process, making phenol degradation a necessary step in the wastewater treatment process. This study was carried out for isolation of phenol degraded bacteria found in waste water. In a study of Mohite et al. [21], from a contaminated soil sample, aerobic Streptococcus
**epidermis** was isolated using enrichment technique on medium containing phenol as carbon and energy source. Many bacterial strains were isolated in pure culture and selected for their ability to degrade phenol. The isolate ABO11 used 0.8 g/l phenol in 10 days while lower degradation was obtained by Mohite et al. [21], who stated that their bacterial isolate was able to degrade phenol up to 200 mg/l. As it was well known, phenol is oxidized to catechol by a phenol hydroxylase and catechol is further degraded using catechol 1,2-dioxygenase [22]. Phenotype and genotype characterization of isolated bacterium indicated that the most active isolate was a Gram-positive, motile and rod-shaped bacterium and belonged to genus Bacillus. Sequencing of the 16S rDNA gene shared 95% identity with that of genus Bacillus. The xenobiotics tolerant strain Bacillus sp. has been isolated from soils contaminated with xenobiotics and currently used for bioremediation [23]. Hasan and Jabeen [24] obtained *Pseudomonas* sp. (IES-S) and *Bacillus subtilis* (IES-B) from contaminated area for malathion, cypermethrin and phenol degradation and they found that the isolates possess high phenol tolerance up to 400 - 700 mg/l phenol without any significant inhibition, hence could be used for phenol remediation, found in wastewaters.

Biodegradation of phenol by bacteria is widely applied for the degradation of phenolic compounds through a specific mechanism and at least two enzymes are involved in the process. Phenol and other phenolic compounds are common constituents of many industrial effluents which can be safely treated and disposed by microbial degradation [25]. From the effluent of the Exxon Mobile Oil Refinery waste water treatment and under aerobic condition, *Candida tropicalis* RETLCrl was isolated for phenol degradation [19] while degradation of phenol and some of its alkyl derivatives were found under both aerobic and anaerobic conditions in seven Japanese soil samples [26].
Moreover, *Klebsiella oxytoca* degraded 75% of initial phenol concentration (100 ppm) within 72 hr [27]. Stimulation the growth conditions enhanced growth to degrade very high concentrations of phenol. In this study, the best growth in phenol containing medium was at 40°C, pH 8 and using NH₄Cl as nitrogen source which considered the simplest inorganic nitrogen source for bacterial growth and enzyme and protein synthesis. *Actinobacillus* species degraded phenol and achieving the higher growth and percentage of phenol degradation at pH 7, incubation temperature of 35°C - 37°C, 150 rpm and using succinic acid and glycine as carbon and nitrogen source [28]. The phenol degradation could be further enhanced by the process of cell immobilization [29] [30]. The effective treatment of phenolic paper factory effluent was obtained using immobilized *Alcaligenes* sp. [31].

5. Conclusion

In conclusion, the selected isolate, *Bacillus steroothermophilus* ABO11 can be used in cleaning the environment from phenol pollution.

References


M. M. A. Aburas


Submit or recommend next manuscript to SCIRP and we will provide best service for you:

Accepting pre-submission inquiries through Email, Facebook, LinkedIn, Twitter, etc.
A wide selection of journals (inclusive of 9 subjects, more than 200 journals)
Providing 24-hour high-quality service
User-friendly online submission system
Fair and swift peer-review system
Efficient typesetting and proofreading procedure
Display of the result of downloads and visits, as well as the number of cited articles
Maximum dissemination of your research work

Submit your manuscript at: http://papersubmission.scirp.org/
Or contact aim@scirp.org