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# Isolation, Selection and Biodegration Capability Investigation of Bacteria Chemotactic to Toluene

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**Abtract**: Aromatic compounds resulted from untreated industrial waste is one of the causes of environmental pollution. The current treatment technology for these compounds is often expensive, complicated or not thorough, causing secondary pollution. Using bacteria chemotactic to pollutants in the detection and treatment of pollutants is a potential treatment measure that offer low cost and simple procedures. In this study, we aim to screen for bacterial strains capable of being chemotatic to toluene, as a model aromatic compound, and degrading it. The experiments testing the chemotaxis of bacteria to toluene were carried out on 0.2% agar minimal medium. The capability of tested bacteria in degrading toluene was experimented on minimal medium solid and semi-solid agar plates. We successfully isolated a strain, designated as HTD 3.12, from soil samples at Tam Dao National Park, that is attractively chemotactic to toluene and also capable of degrading it. The toluene-degrading ability of HTD 3.12 appeared to be more efficient due to its chemotatic activity to toluene.

*Keywords:* Bacterial chemotaxis, toluene, biodegradation.

## 1. Introduction

Waste disposal problem is a matter of worldwide concern. Among the waste products causing environmental pollution, aromatic compounds such as styrene, toluene, DDT,... are persistent and require proper treatment [1]. They can be generated from industrial activities such as oil extraction, machine building, metallurgy, or even in the daily life activities of people such as littering, using plastic bags, dyeing, using detergents [2] ... Long-term accumulation of these compounds accumulate in soil and water can lead to environmental pollutions, as they can cause respiratory irritations or cancer [3].

The detection of aromatic compounds in the environment has been based on many methods such as chromatography, optical sensors or using commercial kits [4, 5]. These methods are highly effective but quite complicated and expensive. Using biological methods is a promising solution that can overcome those disadvantages [6]. Recently, many biological studies also aim to explore the possibility of exploiting microbial chemotaxis for detection and also treatment of aromatic pollutants [7]. Chemotaxis in bacteria is their ability to move to meet the chemical stimuli under the influence

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of the concentration gradient [8, 9, 10]. It is believed that chemotaxis can aid and improve biodegradation by microbes. Therefore, in this study, we screened for bacteria that are chemotactic to toluene, a model aromatic chemical, and also investigate their capability in degrading this compound.

## 2. Materials and Methods

### *2.1. Microorganisms*

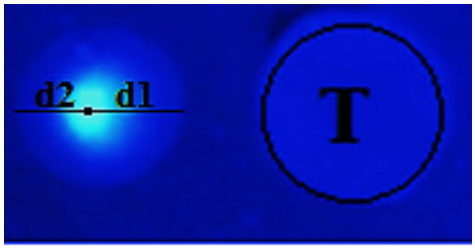
Bacterial strains isolated from soil samples in the following national parks: Tam Đảo, Cúc Phương and Xuân Thủy. Isolates were stored in LB (Luria Bertani) agar slant tube containing 0.5% NaCl and monthly transplanted.

### *2.2. Chemicals and media*

Experiments were conducted in minimal medium composed of: 2 g agar (0.2% agar), 5 g NaCl, 1,47 g $K_2SO_4.3H_2O$, 0.48 g $KH_2PO_4$, 0.132 g $(NH_4)_2SO_4$ in one liter. After sterilization at 121ºC and 1 atm, the medium was supplemented with the following components to their final concentrations: 1 mol/l $MgSO_4$, 1 mol/l Thiamin HCl, 1 mol/l Glycerol.Toluene (Xilong, China) was mixed with 1% agarose to the concentration to be tested.

### *2.3. Chemotaxis tests*

The isolated bacteria were tested for their mobility and then their chemotatic ability in soft agar (0.2% agar) with an agar plug containing toluene placed onto the center of each agar plate. The cells of the bacteria to be tested were stabbed to each agar plate at a distance of 2 cm to the center of the plate. The plates were incubated for 24 hour at 30°C. The chemotaxis index **i** was used to evaluate chemotactic ability of the tested bacteria. **i** is calculated as follows: assuming that a bacterial colony swarm in the soft agar in response to the chemical (toluene) and has the following shape:

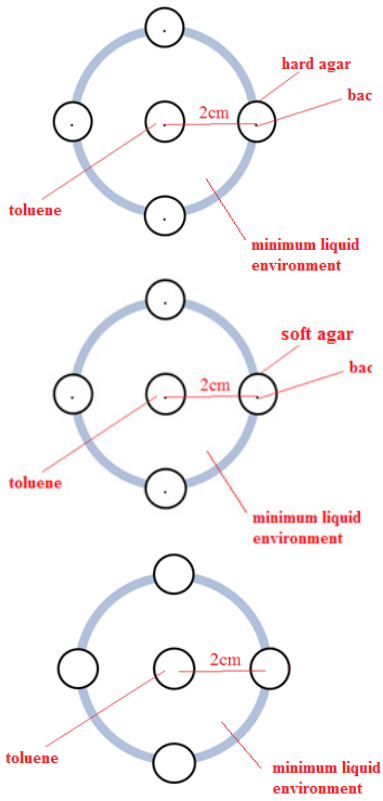



where as: d1: the distance from the center of the colony to the closest edge to the chemical; d2: the distance from the center of the colony to the furthest edge to the chemical

Chemotaxis index is calculated as:

$$i = \frac{d1}{d2} - 1$$

If i>0, the chemotaxis is positive; if i<0, the chemotaxis is negative.




Case 1.The motility and chemotaxis of bacteria is inhibited, as they are inoculated on a plug containing 1.6% agarose.

Case 2. Chemotactic bacteria can function normally because they are inoculated on a soft agar plug.

Case 3. (Control) No bacteria are inoculated.

Figure 1. The experiment design of the degradation test.

Bacterial densities and toluene concentrations were measured over the experimental period.

*Degradation tests*

The selected bacteria chemotactic to toluene was subsequently tested for their ability to degrade toluene in solid, semi-liquid and liquid media containing 1.6%, 0.2% and 0% agarose, respectively. For liquid medium tests, bacterial cell density was determined by measuring the absorbance of the culture with a spectrophotometer at a wavelength of 600 nm; the concentration of toluene was determined by similarly measuring the absorbance of the culture at 266 nm.

The experimental design of the degradation test is described in Fig. 1.

## 3. Result and dicussion

### *3.1. Results*

*a. Selection of bacteria chemotactic to toluene*

We isolated 52 strains of bacteria having the motility, and screened out an active strain, HTD 3.12 (from Tam Đảo National Park), that showed positive chemotatic response to toluene. Toluene concentrations greatly affected the chemotaxis of HTD 3.12. HTD 3.12 showed positive chemotatic responses to the concentrations of toluene higher than or equal to 0.001 M (Fig. 2). Under these conditions, the chemotatic ability of HTD 3.12 was relatively proportional to the concentration of toluene (Fig. 3). At the concentration of toluene of $10^{-6}$ M or lower, the strain was no longer able to sense the presence of toluene in the environment (Fig. 2).

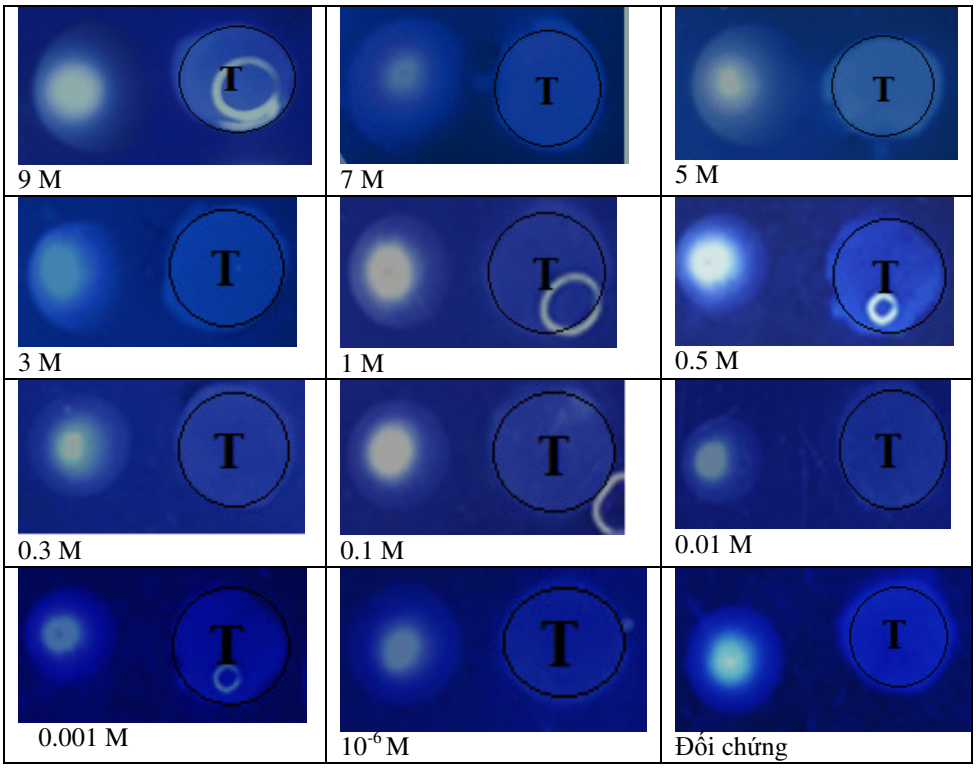



Figure 2. The influence of toluene concentrations on the chemotaxis ability of HTD 3.12.

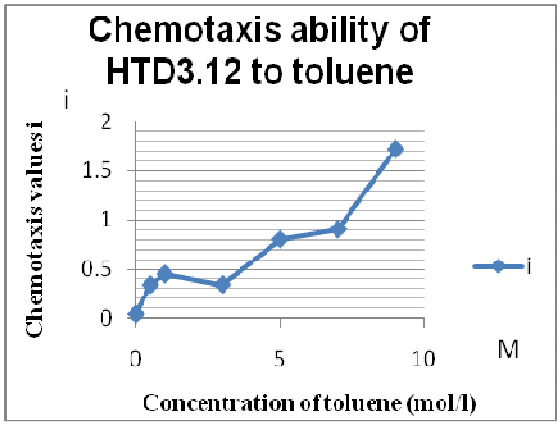



Figure 3. The dependence of the chemotaxis of HTD 3.12 on the concentration of toluene.

From all the results reported above, it can be concluded that ***toluene is a very powerful chemoattractant*** to HTD 3.12.

*b. Degradation*

Case 1: In solid minimal medium containing 1.6% agarose

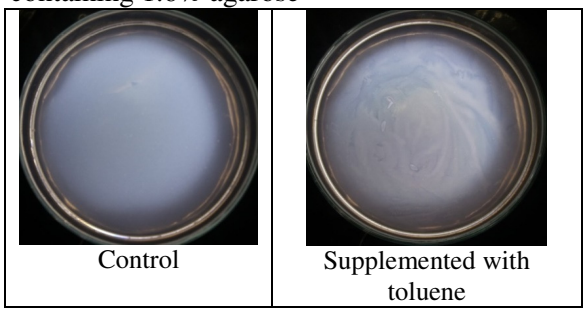



Figure 4. The growth of HTD 3.12 on minimal medium solid agar containing 1M toluene.

The results (Fig. 4) showed that HTD 3.12 could potentially grow in a medium containing only toluene as the carbon source and thus might use toluene as the substrate.

Case 2: In liquid minimal medium containing 0% agarose

HTD 3.12 was cultured while being shaken in a medium containing only toluene as the carbon source. After 7 hours of incubation with only toluene as the carbon source, the cell density of HTD 3.12 increased significantly (Fig. 5). At the same time, the concentration of toluene significantly decreased over time, down to only 30% of the initial concentration after 3 hours of incubation (Fig. 6). Here, in order to sensitively detect the change of toluene concentration, we experimented 5 mM of toluene, because it is the highest concentration of toluene that can be soluble in water.

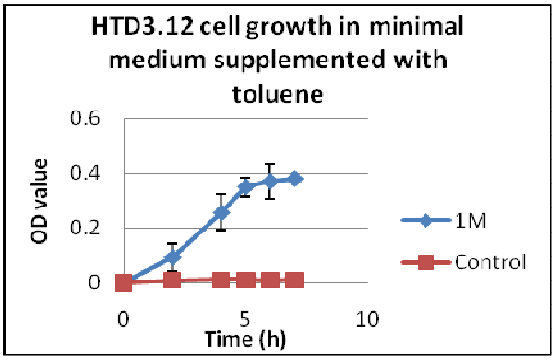



Figure 5. The growth of HTD 3.12 in the presence of toluene as the only carbon source.

Note: 1M: HTD 3.12 cultured in liquid minimal medium containing 1M of toluene; Control: HTD 3.12 cultured in liquid minimal medium without toluene.

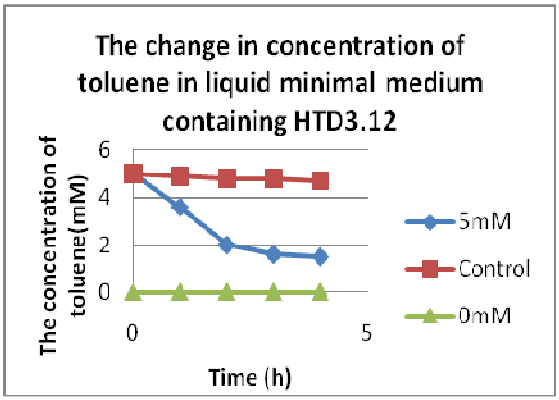



Figure 6. The change in the concentration of toluene over time when HTD 3.12 was cultured in a liquid medium containing only toluene as the carbon source.

Note: 5mM: minimal medium containing 5 mM toluene; 0mM: minimal medium containing no toluene; Control: no bacteria.

Based on the results above, it can be concluded that HTD 3.12 is capable of degrading toluene.

*c. Comparison of the toluene-degrading efficiencies of HTD 3.12 in different chemotactic conditions*

The results obtained (Fig. 7) showed that the toluene concentration naturally decreased over time possibly due to evaporation into the environment (control experiment). However, in the semi-liquid medium, where bacteria have the ability to move and HTD 3.12 can be chemotactic to toluene, the concentration of toluene significantly decreased faster. In the solid medium experiment, the change of toluene concentration was almost similar to that of the control, suggesting that the strain can not access toluene to degrade despite its efficient degradation. These results basically confirmed the advantages of bacterial chemotaxis in aiding bacteria to find and degrade pollutants.

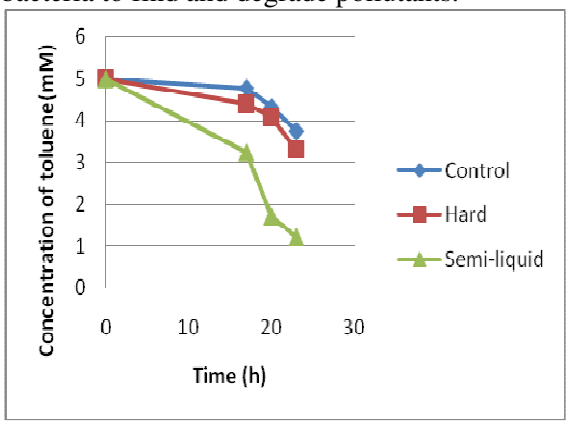



Figure 7. Changes of the concentrations of toluene under different conditions for the chemotactic activity of HTD 3.12.

Note: Control: do not contain bacteria; Hard: experiments with hard agar (bacterial cells can not move); Semi liquid: experiment on soft agar (chemotaxis can be in effect).

### *3.2. Discussion*

The results in this study indicate that bacteria that are chemotactic to and also able to degrade pollutants such as toluene can be obtained from natural environment. It is clear from the results that chemotactic ability enables bacteria to search, access and thus degrade pollutants more efficiently. This is in the same line with some concepts proposed elsewhere about the roles of bacterial chemotaxis in bioremediation [7].

The finding of this study is a significant premise for further developments of biological measures to address environmental pollution that are more effective, cost-saving and environmentally friendly.

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# Phân lập, tuyển chọn và đánh giá khả năng phân giải sinh học của vi khuẩn có hoạt tính hóa hướng động với toluen

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**Tóm tắt:** Các hợp chất thơm có nguồn gốc từ chất thải công nghiệp chưa qua xử lý là một trong những nguyên nhân gây ô nhiễm môi trường. Công nghệ xử lý hiện nay đối với các hợp chất này thường là tốn kém, phức tạp hoặc không triệt để, gây ô nhiễm thứ cấp. Sử dụng vi khuẩn có khả năng hóa hướng động tới các chất ô nhiễm trong việc phát hiện và xử lý là một biện pháp tiềm năng mang lại chi phí thấp, thực hiện đơn giản. Trong nghiên cứu này, chúng tôi sàng lọc các chủng vi khuẩn có khả năng hóa hướng động đến toluene, là hợp chất thơm điển hình và nghiên cứu khả năng phân giải toluene của chủng này. Các thí nghiệm kiểm tra khả năng hóa hướng động của vi khuẩn đến toluen được thực hiện trên môi trường tối thiểu 0,2% agar. Khả năng phân giải toluen của vi khuẩn đã được thử nghiệm trên đĩa môi trường tối thiểu thạch rắn và bán rắn. Chúng tôi phân lập thành công một chủng HTD 3.12, từ các mẫu đất tại Vườn quốc gia Tam Đảo, đó là vi khuẩn hóa hướng động dương đến toluene và cũng có khả năng phân giải hợp chất này. Khả năng phân giải toluene của HTD 3.12 tỏ ra có hiệu quả hơn nhờ hoạt tính hóa hướng động của vi khuẩn này đến toluen.

*Từ khóa:* Hóa hướng động của vi khuẩn, toluene, phân giải sinh học.