Biodegradation of Phenolic Compounds

Microbial diversity 1997

Acharawan Thongmee

Abstract

The degradation of some phenolic compounds by bacterial community in soil and sediment were studied in this experiment. The used of variety inocula in the different kinds of enrichment media had been performed in this study in order to establish which community has the best capability to degrade gallic acid, β-hydroxybenzoic acid, ferulic acid and syringic acid in both aerobic and anaerobic condition. This experiment showed that sediment enriched in M9 medium in aerobic and anaerobic condition has the great ability to degrade the phenolic compounds of choice completely in 3 days. Furthermore, bacterial community in soil can also degrade these compounds in both aerobic and anaerobic conditions.

Introduction

A major source of environmental pollution has been the manufacturing and release of synthetic compounds for used as herbicides, pesticides, refrigerants or solvents. Aromatic, polyaromatic hydrocarbons and phenolic compounds are industrial chemicals that occur both as products of or intermediates in chemical syntheses. They are also source of considerable environmental pollution. These compounds tend to accumulate in nature, and exert their toxic effects in animals and humans. However, microorganisms in nature which are responsible for removal and transforming of toxic and waste materials into harmless compounds adapt themselves and develop the capability to degrade such compounds.

The biodegradation of phenolic compounds has been studied extensively. The pathway used for degrading these compounds depends on the bacterial strain involved. The ring cleavage mechanism includes hydrogenation of the aromatic nucleus to give the corresponding cyclohexane and subsequent ring fission. Under aerobic conditions, oxygenase participate in ring cleavage result in products such as pyruvate, succinate and acetate. In the absence of oxygen, the cleavage of these compounds can proceed in many ways, for example, by bacterial sulfate reducers, by methanogenesis bacteria and by acetogenic bacteria (Hanselman and Kaiser, 1995, Kaiser and hanselmann, 1982 and Daniel et al., 1988). The mechanism of aromatic degradation involves in the demethylation of methoxy substitutes and subsequent hydration of oxygen moieties to form hydroxy-substituted phenols. In the presence of methanogenic bacteria, the final end products are CO₂ and CH₄, while in the presence of sulfate reducing bacteria the final products are CO₂ and hydrogen sulfide.
Recently several reports show that many microorganisms can degrade phenolic compounds. It is uncertain whether the same organisms are responsible for the initial demethylation of the substituted aromatic ring are also responsible for the whole process of ring cleavage and produce acetate or CO₂. The objective of this project was to study the microbial community that can degrade some phenolic compounds completely under aerobic and anaerobic conditions and to establish the capability of specific organism to carry out specific degradation step.

Materials and Methods

Media

Two samples, soil and sediment were selected as inocula. The base media used in this study were

1. M9 medium contained (in g/l) Na₂HPO₄, 6 g; KH₂PO₄, 3 g; NH₄Cl, 1 g NaCl, 0.5 g; 2 ml of 1 M MgSO₄·7H₂O; 0.1 ml of 1 M CaCl₂,

2. Burk's medium contained (in g/l) KH₂PO₄, 0.16 g; K₂HPO₄, 0.64 g; MgSO₄·7H₂O, 0.2 g; CaSO₄·2H₂O, 0.05 g; NaCl, 0.05 g; FeSO₄·2H₂O, 0.003 g; Na₂MoO₄·2H₂O, 0.001 g;

3. Dialysed soil medium was made by placing 10 g of finely soil in water-washed dialysis tubing and placing it inn 50 ml of distilled water contained in 250 ml Erlenmeyer flasks. These were sterilized by autoclave at 121 C for 15 minutes.

All the autoclaved base media were added with 2 mM syringic acid, 2 mM β-hydroxybenzoic acid, 2 mM gallic acid, 2 mM ferulic acid which were sterilized by filtration.

Initial enrichment

For aerobic condition, one gram of each inoculum was inoculated in 50 ml of each medium in 250 ml flask and incubated at room temperature with shaking 200 rpm. For anaerobic condition, one gram of each inoculum was inoculated in bottles gassed with N₂, and incubate the same temperature without shaking.

Secondary enrichment

After observing the turbidity, the primary enrichment (10% V/V) was transferred to the same medium and streak for the isolation attempts on the solid media which has the same constituents, and then incubated in the same condition.

Analytical methods

1 ml of each enrichment was collected for determination phenolic compound by HPLC every three days. Syringic acid, vanillic acid, gallic acid, β-hydroxybezoic acid, catechol and ferulic acid were analyzed by HPLC on Water LC Module 1 equipped with a UV detector set at 254 nm. The flow rate was 0.2 ml/min, the column was a Water Bondapak C18 column (length 3.9 x 150 mm, 125 A). A mixture of methanol: H₂O: acetic acid in the ratio of 19:76:5 adjusted to a pH of 2.6 was used as eluent. Elution times and the concentrations were calibrated using standards of known concentrations.
Microscopy

Bacteria were examined under microscope using wet mount technique after the turbidity was observed in the liquid enrichment. The presence of methanogenic bacteria was tested by using the blue fluorescence of the F420 cofactor.

Identification of the pure culture

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**Fig 1** The overall diagram of the experiment
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Results

Growth
Growth was observed in every enrichments. Therefore, the culture was inoculated on the agar media to get the isolated colonies. After one week of incubation, small colonies appeared on the plates. The isolate colonies were transferred in order to get the pure culture.

Phenotypic characterization
The enrichment contained short rod, motile bacteria and fungi (fig2). One of the isolate colonies characterize as Gram-negative bacteria which have the enzyme oxidase. However, the 16SrRNA sequencing failed

HPLC analysis of biodegradation
Fig3 and fig4 summarized the analytical results using HPLC. All enrichments showed decomposition of some phenolic compounds.

In aerobic condition, the enrichment of soil and sediment in M9 medium in aerobic condition showed the complete degradation of gallic acid and β-hydroxybenzoic acid within 3 days of incubation. The enrichment of soil and sediment in burk’s medium and dialysed soil medium also showed the degradation of the same compounds but the rate of degradation are slower than the enrichment in M9 medium. Syringic acid was degraded in a slower rate when compared to other compounds. Catechol was observed during the degradation of gallic acid, ferulic acid and β-hydroxybenzoic acid, so it was thought to be one of the intermediate product in the degradation pathway.

In anaerobic condition, the rate of decomposition of gallic acid, β-hydroxybenzoic acid, and ferulic acid was slower than the decomposition of the enrichment in aerobic condition. No catechol was observed during the degradation process.

Discussion and conclusions

The experiment showed that bacteria enriched in this experiment can grow on phenolic compounds and use these compounds as the carbon source and energy source. Gallic acid and β-hydroxybenzoic acids were the first compounds that were degraded by the bacteria community in this soil and sediment under aerobic condition. Therefore, these compounds were used as sole of carbon and energy source. As described by Reusch and Šdoff (1981), they showed that hydroxybutyrate was usually directed into central metabolism through the generation of acetyl-coenzyme A, and also has been shown to be the precursor of novel lipids essential for encystment of Azotobacter vinelandii. In anaerobic condition, this study show that some of syringic acid was degraded even though the rate of degradation was very slow. It was established that Anaerobic microbial community was able to degrading syringic acid compleatly to CH₄ and CO₂ (Kaiser and Hanselmann 1982). The community can be maintain with the syringic acid as sole of carbon and energy source. Syringic acid is converted stoichiometrically according to

\[ C₉H₁₀O₅ + 4H₂O \rightarrow 41/2 CH₃COOH + 41/2CH₄ + 41/2CO₂ \]
It was confirmed that the degradation of the compounds is catalyzed by the organisms because there was no significant change in the amount of phenolic compounds in negative controls (contain the same medium but no inoculum).

References


Fig 8.
Fig 4.
ISOLATION OF HALOPHILIC BACTERIA FROM THAI FISH SAUCE AND DRIED SQUID

Acharawan Thongmee
Microbial diversity 1997

Objective
To cultivate, isolation and characterization of halophilic bacteria from fish sauce and dried squid.

Hypothesis
There may be some halophilic bacteria in fish sauce and dried squid.

Introduction
Thai fish sauce
: prepared from fish in concentrated brine.
: clear, brown and salty liquor.
: an important source of amino nitrogen, protein, iron, calcium and vitamins.
: two parts fish (Stolephorus spp.) + one part marine salt
  + concentrated brine (4.4-5.1 M NaCl)

Dried squid
: prepared from squid
: preserved with salt (3-4 M NaCl)
: air dried with sunlight
50 g. dried squid + 450 ml. HB

\[ \text{blender} \]

\[ \text{supernatant} \quad \text{Fish sauce} \]

\[ \text{enrichment culture in HB (10, 15, 20, 25\% \ NaCl)} \]

\[ \text{turbidity} \]

\[ \text{serial dilution (10}^1, \text{ 10}^2, \text{ 10}^3, \text{ 10}^4) \]

\[ \text{spread on HA (10, 15, 20, 25\% \ NaCl)} \]

\[ \text{observe growth} \]

**Halophile media**

- MgSO$_4$ 7H$_2$O 2\%
- casamino acid 1\%
- yeast extract 1\%
- proteose peptone 0.5\%
- trisodium citrate 0.3\%
- Kcl 0.2\%
- NaCl 10, 15, 20, 25\%
- pH 7.2 ±0.2
Results

Table 1  Enumeration of bacteria on halophile agar with 10, 15, 20, 25% NaCl (viable count).

<table>
<thead>
<tr>
<th>specimen</th>
<th>salt concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>dried squid</td>
<td>1 x 10^2</td>
</tr>
<tr>
<td>fish sauce</td>
<td>-</td>
</tr>
</tbody>
</table>

Characteristics

A: Gram-negative rod, motile, 2.5 m in length and 0.3 m in diameter.
   Colony morphology: round, 1-2 mm. in diameter, convex, smooth, white
   → From its tRNA, it is *Halo bacillus litoralis*

B: Gram-positive cocci, 1-1.5 min diameter
   Colony morphology: round, 1-2 mm. in diameter, smooth, white
   Catalase positive → *Staph. spp.*

C: Small colonies 1 mm in diameter, round, convex and translucent.
Halophilic bacteria isolated from fish sauce and dried squid.