Isolation of di(2-ethylhexyl)phthalate degrading bacteria from Antarctica soil flora

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1. Background

Environmental pollution caused by plasticizers in plastics has become a problem. Plasticizers are persistent and they are known to have an adverse effect on ecosystems when eluted from plastics into the environment.

Di(2-ethylhexyl)(DEHP)

- The most used plasticizers
- Reported to cause reproductive dysfunction
- Difficult to recover or remove
- Biodegradable by microorganisms

Bioremediation

The method to clean up environmental pollution by utilizing the ability of microorganisms to degrade chemical substances

Advantages

- Inexpensive and extensive purification
- Low environmental impact

Disadvantage

- Dependent on environmental conditions
- Difficult to clean up high concentrations of pollution

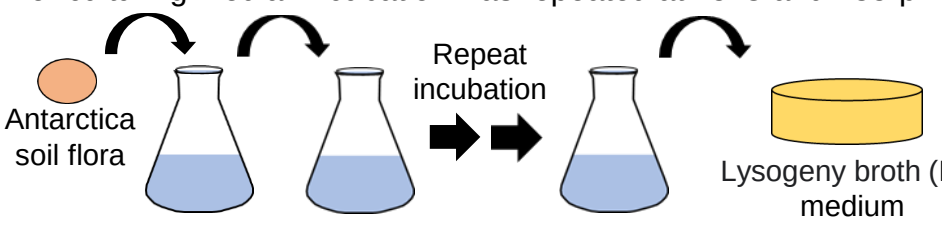
Objectives

- **To isolate DEHP-degrading bacteria from Antarctic soil**
- **To evaluate its biochemical properties and determine its species**
- **To measure its ability to degrade DEHP and hydrolytic enzyme activity**

2. Materials and Methods

2-1. Isolation of bacterium

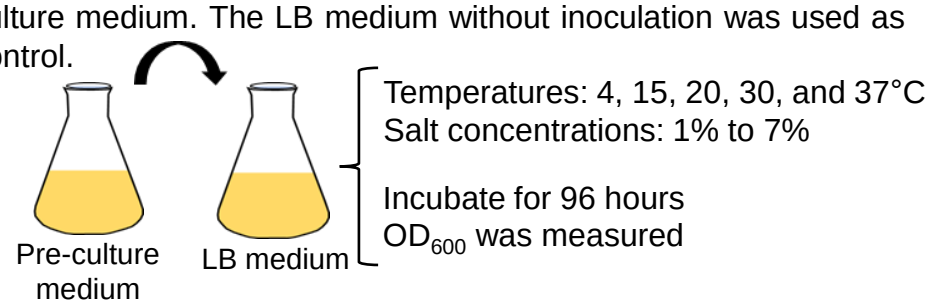
Mineral salt medium (MSM) containing 1% (v/v) DEHP was used for culturing media. Incubation was repeated at 15°C and 135rpm.



2-2. Biochemical properties of strain HP5

Growth temperature and Salt tolerance test

Incubate strain HP5 in LB medium for 48 hours to prepare pre-culture medium. The LB medium without inoculation was used as control.

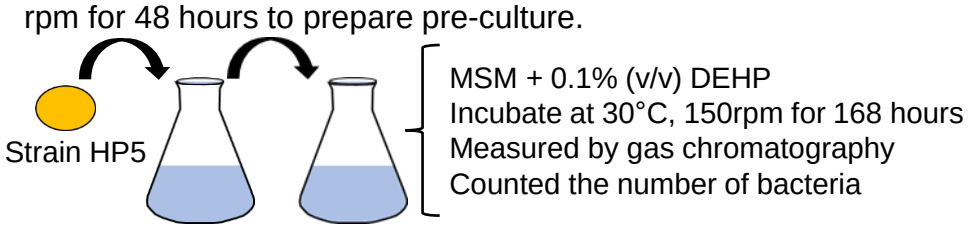


2-3. 16S rRNA sequence of strain HP5

The genome DNA of strain HP5 were extracted and its 16S rRNA sequence was amplified by PCR and sequenced.

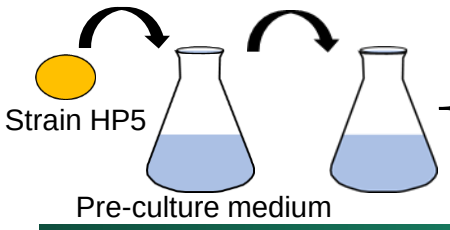
2-4. DEHP degradation test

Incubate strain HP5 in MSM containing 1% (v/v) DEHP at 30°C, 150 rpm for 48 hours to prepare pre-culture.



2-5. Hydrolytic enzyme activities test

The amount of enzyme that produces 1µmol of p-nitrophenol (p-NP) per minute is defined as 1 U.



3. Results and discussion

3-1. Isolation and identification of strain HP5

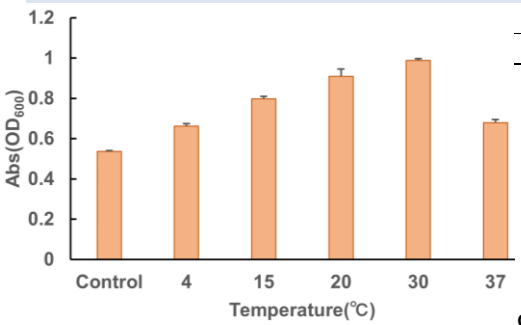


Fig.1 Bacterial volume

Table.1 Bacterial properties

	Strain HP5	*R. erythropolis*
Gram stain	+	+
Shape	Rod	Rod
Catalase	+	+
Oxidase	-	-
Motility	-	-
Indol	-	-
Hydrogen sulfide	-	-
IPA	-	-
Optimal temperature	30℃	28℃
Solt concentration	6%	5%

Strain HP5 even grew at low temperatures such as 4 and 15°C (Fig.1). The result of 16SrRNA sequencing showed that the strain HP5 is the highest similarity (99.25%) with *Rhodococcus erythropolis* (NCBI:txid1833). Bacterial properties of strain HP5 and *R. erythropolis* are shown (Table.1).

3-2. DEHP degradation test

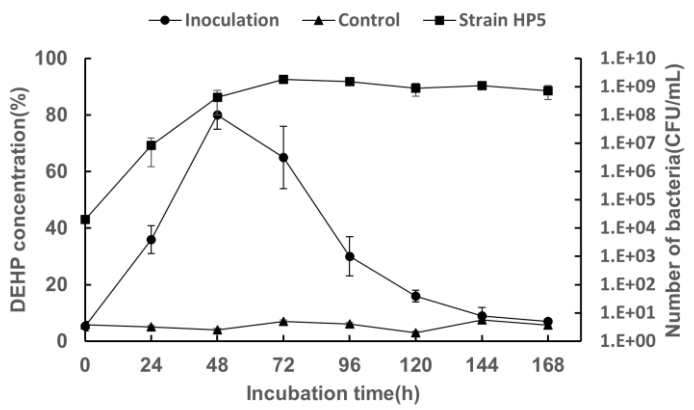


Fig.2 DEHP concentration and Number of bacteria in culture

DEHP concentration in culture was about 6.9% after 168 hours (Fig.2). Since DEHP was not degraded and dispersed in the medium, DEHP concentrations of control were low.

The number of bacteria reached a maximum at 72 hours and the generation time was 4.5 hours.

3-3. Hydrolytic enzyme activities test

The result of hydrolytic enzyme activities test showed esterase activity of 0.0325 U/mL.

These results showed that strain HP5 could metabolize DEHP as carbon source for bacterial growth and DEHP was degraded by esterase.

3. Discussions and Conclusions

Strain HP5 isolate from Antarctic soil flora has very similar biochemical properties to *R. erythropolis* and is likely to be the same species as it.

Strain HP5 degrades DEHP at higher concentrations, although at a slower degradation rate than the reported bacteria[1)].

Strain HP5 is a cold-tolerant bacterium and has excellent salt tolerance, which may contribute to bioremediation in the ocean.

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