Evaluation of Adaptive Laboratory Evolution (ALE) Using the *Comamonas* sp. C10 Strain for the Enhancement of Polyhydroxybutyrate (PHB) Degradation Capability

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Goals

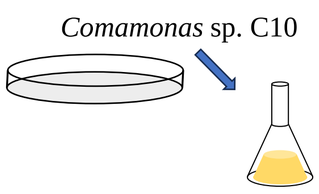
Improving the PHB (polyhydroxyalkanoate) degradation capability through PHB-degrading bacteria is considered a potential aid in addressing the biodegradation challenges associated with PHB, which is used as an alternative to petroleum-derived plastics. Therefore, in this study, we investigated the enhancement of PHB degradation capability in the *Comamonas* sp. C10 strain using Adaptive Laboratory Evolution (ALE).
Through ALE, we incrementally increased the PHB concentrations to 0.2%, 0.5%, and 0.8%. At each concentration, when a degradation rate of 80% or more was observed, we proceeded to the next higher concentration of PHB in the culture medium for further degradation experiments, conducting adaptive cultivation. At the maximum concentration of 0.8%, we compared the degradation efficiency between strains induced by ALE and strains without induction, evaluating the effectiveness of ALE.

Experimental Concept

Plastic alternatives;
Polyhydroxybutyrate: PHB
Degradation
Comamonas sp. C10
Enhancement of degradability
→ Towards the resolution of biodegradation issues

Methodology

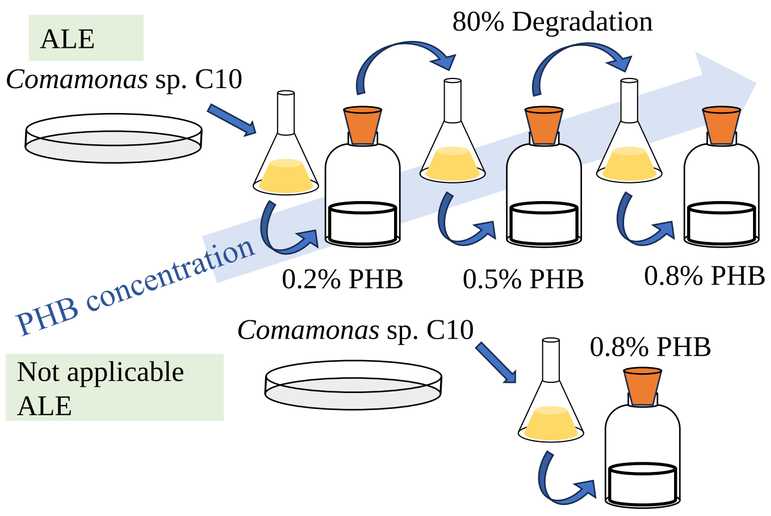
1. Determination of optimal pre-cultivation time
2. Determination of maximum concentration
3. Improvement experiment of degradation capability



Comamonas sp. C10
LB liquid medium 100 mL

Measurement of optical density (OD_{600}) at each hour for 24 hours.

Sampling
↓
Cleaning and drying PHB polymer
↓
Alkaline treatment of the diried samples
↓
Measurement of PHB concentration by High-Performance Liquid Chromatography (HPLC) analysis
↓
Subculture when the degradation rate reaches 80% or higher



Sampling
↓
Cleaning and drying PHB polymer
↓
Alkaline treatment
↓
Measurement of PHB concentration by High-Performance Liquid Chromatography (HPLC) analysis
↓
Comparison of degradation rates at the maximum concentration (0.8%)

Results

1. Determination of pre-cultivation time

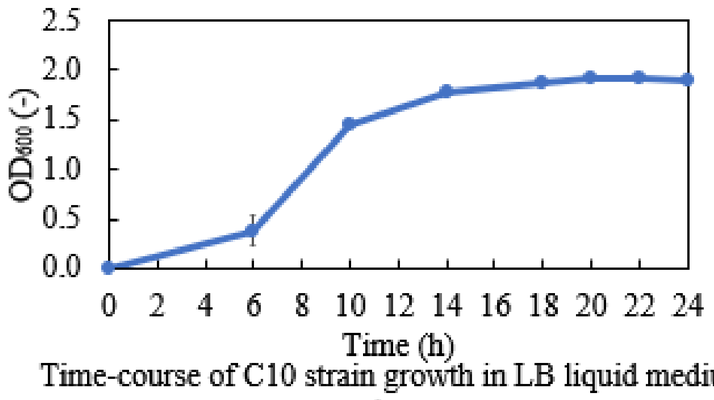


Fig. 1 Time-course of C10 strain growth in LB liquid medium. The cultivation conditions were 30℃ and 100 rpm. Data represent the average of two experiments.

2. Determination of maximum concentration

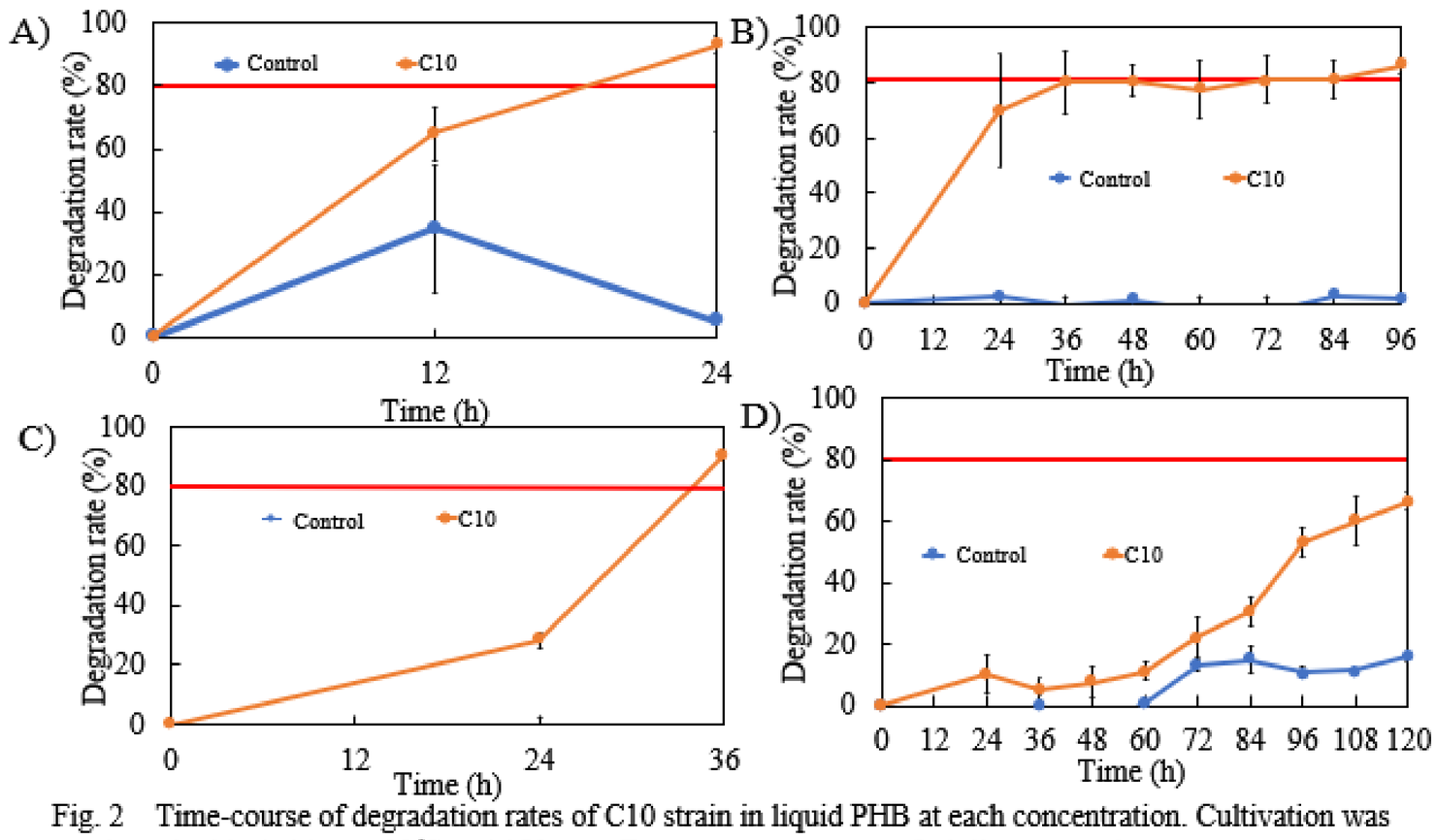


Fig. 2 Time-course of degradation rates of C10 strain in liquid PHB at each concentration. Cultivation was conducted with shaking at 30℃ and 150 rpm. Data represent means of triplicate experiments.
A : 0.2%, B : 0.5%, C : 0.8%, D : 1.0%

3. Degradation capability improvement experiment

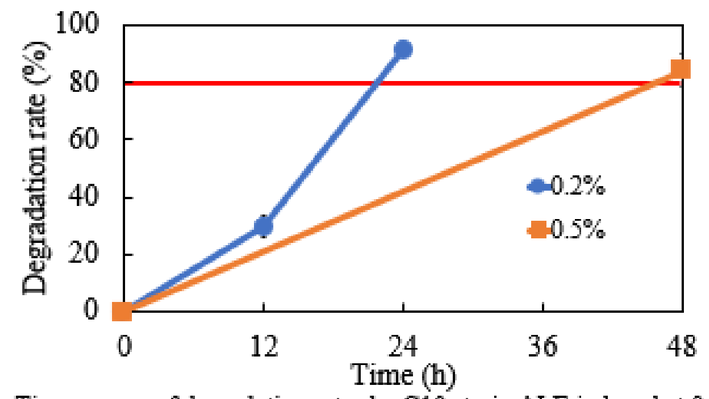


Fig. 3 Time-course of degradation rates by C10 strain ALE induced at 0.2% and 0.5%. Cultivation was conducted with shaking at 30℃ and 150 rpm. Data represent means of triplicate experiments.

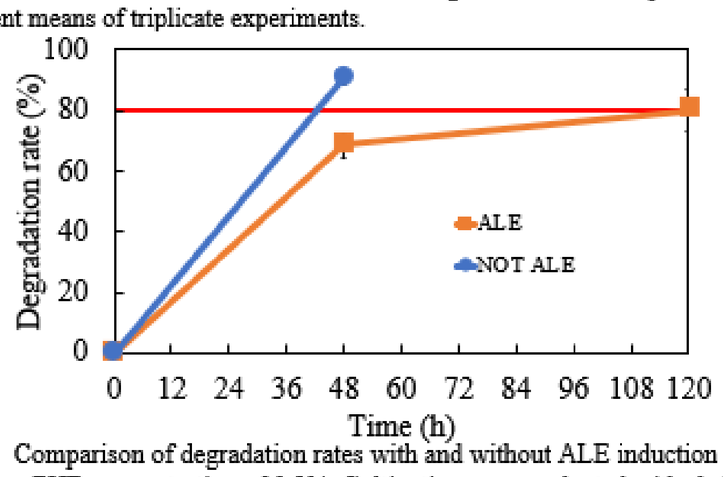


Fig. 4 Comparison of degradation rates with and without ALE induction at the maximum PHB concentration of 0.8%. Cultivation was conducted with shaking at 30℃ and 150 rpm. Data represent means of triplicate experiments.

Conclusions

- The pre-cultivation time for *Comamonas* sp. C10 strain was determined to be 18 hours.
- When ALE was used, it was found that the maximum PHB concentration for *Comamonas* sp. C10 strain to degrade was 0.8%.
- The reason for the faster degradation rate in the absence of ALE induction at 0.8% PHB concentration was due to the fact that under the ALE-induced condition, the strain was directly inoculated from LB liquid medium containing pre-cultivation with 80% degradation observed of 0.5% PHB (weak cell activity), while under the non-ALE-induced condition, the inoculum was obtained from fresh colonies formed on 0.1% PHB agar medium.
- This suggests that the bacteria in the ALE-induced medium might have already undergone high-concentration PHB degradation, potentially leading to a reduced degradation rate in the subsequent culture.

- In the near future, the ALE-induced medium will be transferred to 0.1% PHB agar medium once, and newly formed colonies will be used for pre-cultivation. This allows for control using bacteria between those induced by ALE and those not induced. Both the same growth condition and the same composition medium allow a comparison of the effectiveness of ALE.