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The Study of the Microbes Degraded Polystyrene

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Abstract

Under the observation that *Tenebrio molitor* and *Zophobas morio* could eat polystyrene (PS), we setup the platform to screen the gut microbes of these two worms. To take advantage of that *Tenebrio molitor* and *Zophobas morio* can eat and digest polystyrene as its diet, we analyzed these special microbes with PS plate and PS turbidity system with time courses. There were two strains TM1 and ZM1 which isolated from *Tenebrio molitor* and *Zophobas morio*, and were identified by 16S rDNA sequencing. The results showed that TM1 and ZM1 were cocci-like and short rod shape Gram-negative bacteria under microscope. The PS plate and turbidity assay showed that TM1 and ZM1 could utilize polystyrene as their carbon sources. The further study of PS degraded enzyme and cloning warrants our attention that this platform will be an excellent tools to explore and solve this problem.

Keywords: polystyrene, tenebrio molitor, zophobas morio, 16S rDNA sequencing

1. Introduction

Currently, the most popular method to decompose polystyrene was the thermal decomposition method, but this method would produce large amounts of dioxin and cause serious pollution to the environment [1, 2]. All these thermal methods faced an interaction with the polymer breakdown products which could lead to the formation of well-known dioxin precursors such as halogenated phenols should also be taken into account when evaluating the impact of BFR-treated materials on the environment [1]. However, the recent research directions of polystyrene degradation will be that use the way of biological decomposition, and these methods would reduce the waste polystyrene and dioxin.

The approach of biodegradation of polystyrene was initiated in 1979, all bio-species show a very low degraded ability with C^{14}-labelled plastic[3]. The best result from sludge microbes degraded the polystyrene for 0.57% after 11 weeks. The other species degraded less than 0 to 0.3% after 35 days. Most organisms were not able to degrade PS. To take advantage of PS-eating mealworms, Yang et.al. test the consumption of the PS[4]. Furthermore, they explored the gut of mealworms, and identified of novel bacteria YT2 which belongs to Exiguobacterium sp. and other 12 isolates based on its 16S rRNA sequences[5, 6]. However, their methods for further identification and characterization of PS degrading enzyme of these microbes were not easy.

Our motivation of this study is to establish a more efficient and easy platform to study the PS degraded microbes within these worms. We utilize the mealworms (Tenebrio molitor) as well as superworms (Zophobas morio) for screening the PS eating microbes. Furthermore, the microbes in this study were decomposing more quickly than previous studies. The setup of this novel screening platform will be an excellent tool to explore and solve the further study of PS degraded enzyme.

The aims of this study is to isolate the polystyrene degrading microbes from the gut of T. molitor and Z. morio, and the following methods will be used to exam PS-degrading ability of these microbes[7].

2. Method

2.1. Preparation of PS Emulsion and PS Plate

To prepare the PS emulsion, we dissolve the 7.5g PS powder by 50 mL chloroform. Then transferred this solution to a 250 mL glass bottle with 100 mL basal medium (1.6g K_2HPO_4,1g $(NH_4)_2SO_4$,0.2g KH_2PO_4,0.2g $MgSO_4 \cdot 7H_2O$,0.1g NaCl,0.015g $CaCl_2$,0.01g $FeSO_4 \cdot 7H_2O$/1000 mL) and biodegradable detergent[7]. Next transferred the PS emulsion to 4℃ for 2 days.

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After 2 days, we put the PS emulsion in hood to volatilize the organic solvents.

The preparation of PS agar plate is to transfer 50 mL PS emulsion to a 100 mL glass bottle with 50 mL basal media and 0.75 g agar, sterile for 15 min at 121℃ and pour plate as general agar plates[7].

2.2. Isolation of PS Degrading Microbes

A group of 20 mealworms and superworms separated fed with polystyrene as a sole diet for 3 weeks was collected, and washed out as a gut microbes' suspension with basal medium.

At first these gut microbes' suspension was plating on the basal agar plate incubating for 24 h at 37℃ in aerobic and anaerobic conditions, and the microbes on the basal agar plate are collected. And then transferred the microbes on the basal agar plate to PS plate with yeast extract incubating for 24 h at 37℃ as above conditions.

Finally, the microbes on the PS agar plate are collected and the pure colonies were preserved for the following experiments.

2.3. Bacteria Strain Characterization and 16S rDNA Sequencing

There were two strains TM1 and ZM1 which isolated from Tenebrio molitor and Zophobas morio, and were identified by Gram staining, TSI agar test and 16S rDNA sequencing.

The genomic DNA of the bacteria strains for 16S rDNA sequencing were extracted by genomic DNA extraction kit (Thermo Fisher Scientific).

The 16S rDNA gene was amplifying by 27F-DegL (5′-AGRGTTYGATYMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′)[8]. The sequences were compared with 16S ribosomal RNA sequences on the 16S rRNA database using the Basic Local Alignment Search Tool (BLAST) in NCBI.

2.4. Turbidity Assay

Turbidity assay was a method following the measurement that Chua et.al. used[7]. This method could measure the PS-degrading ability of these microbes, and it was a quantitative indicator of the PS degradation as well.

To quantify PS degrading activity, 1 ml of an overnight liquid culture of TM1 and ZM1 in PS medium was mixed with 20 ml of basal medium containing 0.5 mL 0.75 % PS emulsion and incubated at 37℃ for following time courses. The PS degrading activity was measured spectrophotometrically at 600 nm.

To observe the utilization of PS emulsion and PS degrading microbes' suspension will be at 0.2, 4, 6, 24, 48 h respectively.

3. Results and Discussion

3.1. Isolation and enrichment of PS Degrading Microbes

After 3 weeks, the Tenebrio molitor and Zophobas morio were dissected and then the guts of the worms were prepared for the gut microbes' suspension. The gut microbes' suspension was transferred to the basal agar plate incubating for 24 h at 37℃ with different yeast extract concentration. The result showed that all of the agar plates have many colonies on basal agar (Fig. 1).

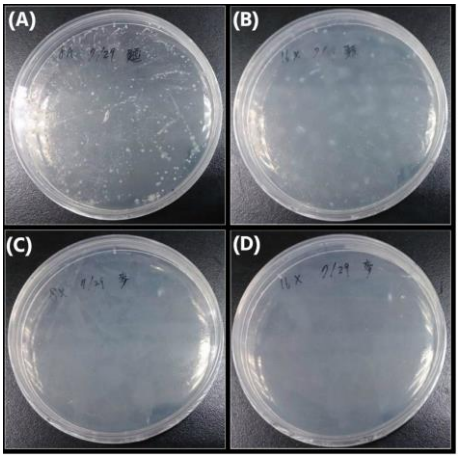

Fig. 1 Gut microbes plated on the basal agar plate (A) Microbes from the gut of *Tenebrio molitor* incubate with yeast extract (0.05g/L). (B) Microbes from the gut of *Tenebrio molitor* incubate with yeast extract (0.1g/L). (C) Microbes from the gut of *Zophobas morio* incubate with yeast extract (0.05g/L). (D) Microbes from the gut of *Zophobas morio* incubate with yeast extract (0.1g/L)

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All of the colonies on the basal agar plate were rinsed with saline and collected. The suspension was plated on the PS plate incubating for 24 h at 37℃ with different yeast extract concentration.

The result showed that all of the agars have white colonies (Fig. 2). The pure colonies were isolated by streaking plate method until the pure colonies were obtained (Fig. 3).

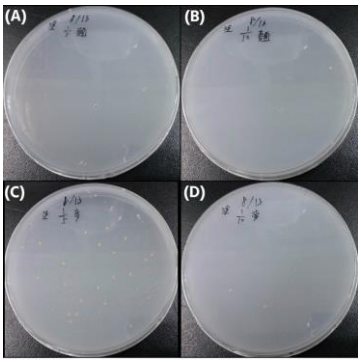

Fig. 2 Gut microbes plated on the PS agar plate (A) Microbes from the gut of *Tenebrio molitor* incubate with yeast extract (0.1g/L). (B) Microbes from the gut of *Tenebrio molitor* incubate with yeast extract (0.05g/L). (C) Microbes from the gut of *Zophobas morio* incubate with yeast extract (0.1g/L). (D) Microbes from the gut of *Zophobas morio* incubate with yeast extract (0.05g/L)

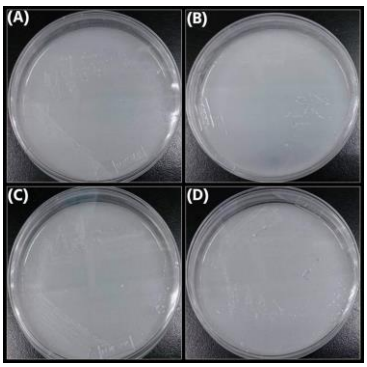

Fig. 3 Pure colonies were isolated by streak plate method (A) Pure colonies of TM1 incubate at anaerobic condition. (B) Pure colonies of TM1 incubate at aerobic condition. (C) Pure colonies of ZM1 incubate at anaerobic condition. (D) Pure colonies of ZM1 incubate at aerobic condition

To confirm that our PS plate could grow only the PS degrading microbes, we cultured different bacteria on PS plate and found that only the microbes which have the ability of PS degradation could grow on the PS plate (Fig. 4). The final result showed that only the TM1 and ZM1 can grow on the PS plate.

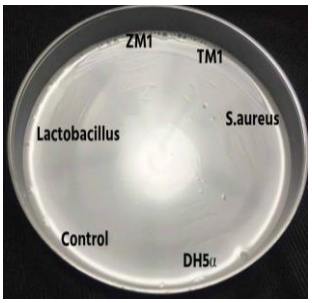

Fig. 4 The PS plate culture with different bacteria: ZM1, TM1, *S. aureus*, DH5 α (*E. coli*), Lactobacillus

3.2. Turbidity assay for the PS-Degrading Ability of the TM1 and ZM1

The colonies from two strains TM1 and ZM1 were added to the PS emulsion to confirm their ability of PS degrading. (Fig. 5). The result showed that the degrading ability of TM1 and ZM1 with yeast extract had more degrading activities than which that without yeast extract.

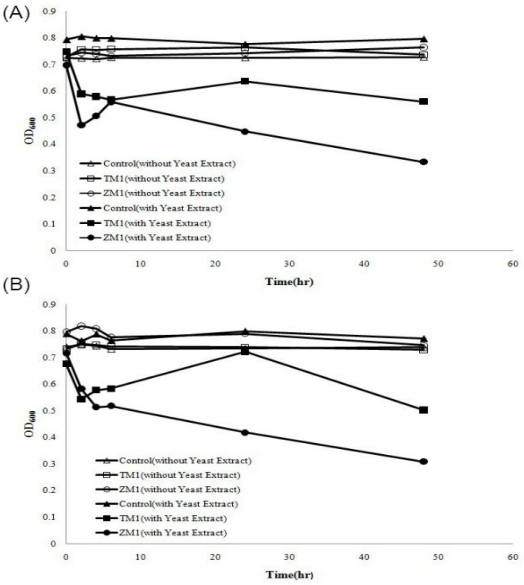

Fig. 5 Turbidity Assay (A) Turbidity Assay of TM1, ZM1 at aerobic condition with or without yeast extract (0.5g/L) (B) Turbidity Assay of TM1, ZM1 at anaerobic condition with or without yeast extract (0.5g/L).

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3.3. Bacteria Strain Characterization and 16S rDNA Sequencing

By Gram staining, the TM1 and ZM1 are showed both Gram negative bacteria. And in the result of TSI agar test, we can confirm that the TM1 and ZM1 are not the same bacteria strain (Table 1). From TSI agar result, the TM1 could be Alcaligenes sp., Pseudomonas sp., or Acinetobacter sp., and ZM1 could be Klebsiella pneumoniae. Howerver, from 16S rDNA data, the TM1 could be Aeromonas sp., and ZM1 could be Klebsiella pneumoniae (Fig. 6). This final confirmation of TM1 strain needs more accurate identification of other method.

Table 1 The results of TSI Agar Test

	TM-1	**ZM-1**
Slant	Alkaline	Acid
Butt	Alkaline	Acid
H_2S	No	No
Gas production	No	Yes
Possible species	Alcaligenes Pseudomonas Acinetobacter	Klebsiella

(A) Description	**Max score**	**Total score**	**Query cover**	**E value**	**Ident**	**Accession**
Aeromonas taiwanensis strain A2-50 16S ribosomal RNA gene, partial sequence	928	928	92%	0.0	84%	NR 116585.1
Aeromonas sanarellii strain A2-67 16S ribosomal RNA gene, partial sequence	922	922	92%	0.0	84%	NR 116584.1
Aeromonas dhakensis strain P21 16S ribosomal RNA gene, complete sequence	922	922	92%	0.0	84%	NR 042155.1
Aeromonas hydrophila strain DSM 30187 16S ribosomal RNA gene, complete sequence	922	922	92%	0.0	84%	NR 119190.1
(B) Description	**Max score**	**Total score**	**Query cover**	**E value**	**Ident**	**Accession**
Klebsiella pneumoniae strain DSM 30104 16S ribosomal RNA gene, partial sequence	1857	1857	99%	0.0	99%	NR 117683.1

Fig. 6 The BLAST results of 16s rDNA sequencing.(A) The BLAST result of 16s rDNA sequencing of TM1(B) The BLAST result of 16s rDNA sequencing of ZM1

4. Conclusions

In this paper, we setup the PS degrading screening platform, which successfully select the TM1, ZM1 from worms' gut. The characteristic of TM1 and ZM1 have been identified through Gram staining, TSI agar test, 16s rDNA sequencing.

This will be the first reported strain from the mealworm and superworm. In the turbidity assay, we can first time show that yeast extract will be a very important co-factor for the TM1 and ZM1 with more efficient PS degrading ability.

The further study of PS degraded enzyme and cloning from TM1 and ZM1 warrants our attention that this platform will be an excellent tools to explore and solve this problem.

Acknowledgement

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