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Filter Paper Degradation by Bacteria Isolated From Local Termite Gut

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Abstract: Bacterial strains isolated from the gut of the local termite *Coptotermes curvignathus* were inoculated into a buffered medium containing minerals and Whatman filter paper as the sole carbon source to observe the ability of the bacteria to digest solid substrate. The bacteria were *Bacillus cereus* strain Razmin A, *Enterobacter aerogenes* strain Razmin B, *Enterobacter cloacae* strain Razmin C, *Acinetobacter* strain Raminalimon and *Chryseobacterium kwangyangense* strain Cb. The Gen Bank NCBI/EMBL accession numbers for the bacterial strains were EU294508, EU305608, EU305609, EU332791 and EU169201, respectively. The ability of bacterial cultures to grow in this medium as well as to digest the filter paper was determined by visual observation after 30 days. All bacterial cultures showed growth as the medium turned cloudy and the filter paper became macerated. *Chryseobacterium kwangyangense* strain Cb showed yellow pigmented colonies on the filter paper. *Bacillus cereus* strain Razmin A showed clumps of degraded filter paper with black dots.

Key words: Cellulolytic bacteria, termite gut, filter paper degradation

INTRODUCTION

There are various species of bacteria in termites gut that are capable of degrading cellulose and hemicellulose (Wenzel *et al.*, 2002). The species include *Bacillus cereus*, *Bacillus anthracis* and *Rhizobium elii*. Cellulolytic bacteria have also been isolated from other insects such silver crickets (Chakraborty *et al.*, 2000). Recently, Ramin *et al.* (2008) isolated a number of cellulolytic bacteria from the local lower termite *Coptotermes curvignathus* gut. However, the cellulolytic activity of these bacterial species on solid substrates like filter paper has not been studied. Therefore, the objective of this study was to observe visually the ability of bacterial strains isolated from the termite's gut to digest filter paper.

MATERIALS AND METHODS

Termites Gut Bacteria

The bacteria used in this study were previously isolated from the gut of the local termite *Coptotermes curvignathus*, collected from the vicinity of Universiti Putra Malaysia (UPM) at March 2008. The bacteria isolated were all novel strains and they were identified as *Bacillus cereus* strain Razmin A (EU294508), *Acinetobacter* strain Raminalimon (EU332791), *Enterobacter cloacae*

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strain Razmin C (EU305609), *Enterobacter aerogenes* strain Razmin B (EU305608) and *Chryseobacterium kwangyangense* strain Cb (EU169201). The cultures were maintained in nutrient agar slant at 4°C.

Medium Preparation and Inoculation

Each bacterial strain was first grown on nutrient agar and incubated at 39°C overnight. Bacterial cell culture was prepared in nutrient broth as inoculum. The medium used for the cellulolytic activity study was as described by Chakraborty *et al.* (2000). The medium contained in g L⁻¹, 2.2 K₂HPO₄; 1.5 KH₂PO₄; 1.3 (NH₄)₂SO₄; 0.1 MgCl₂; 0.02 CaCl₂; 0.001 FeSO₄·7H₂O and the pH was adjusted to 6.9 (with 1M NaOH and HCl). The medium was sterilized at 120°C for 20 min. Seventy five milliliter of the medium was poured into six flasks containing sterilized filter paper as the main carbon source (cellulose) for the bacteria. Five milliliter of each bacterial cell culture adjusted to 0.5 OD₅₀₀ nm was pipetted into the flasks (except the control) and then incubated at 39°C for 30 days.

Growth and cellulolytic activity of bacteria was determined by observing the change in the medium as well on the filter paper. Cloudiness of the medium indicated growth and maceration of the filter paper indicated cellulolytic activity.

RESULTS AND DISCUSSION

Bacterial colonies forming units of each bacterial species on nutrient agar plates are shown in Fig. 1. *Chryseobacterium kwangyangense* strain Cb showed yellowish colonies, while other strains were whitish in colour (Fig. 1). All bacterial species grew well at 39°C. Visual observation of cultures in the medium containing filter paper showed cloudiness which indicated growth and disintegration of the filter paper which indicated cellulolytic activity (Fig. 2). The signs of activity occurred within 10-15 days of incubation and the medium became colloidal after one month. *Chryseobacterium kwangyangense* strain Cb showed the same color of colony (yellow) on filter paper (Fig. 2). *Bacillus cereus* strain Razmin A showed distinct black dots (Fig. 2).

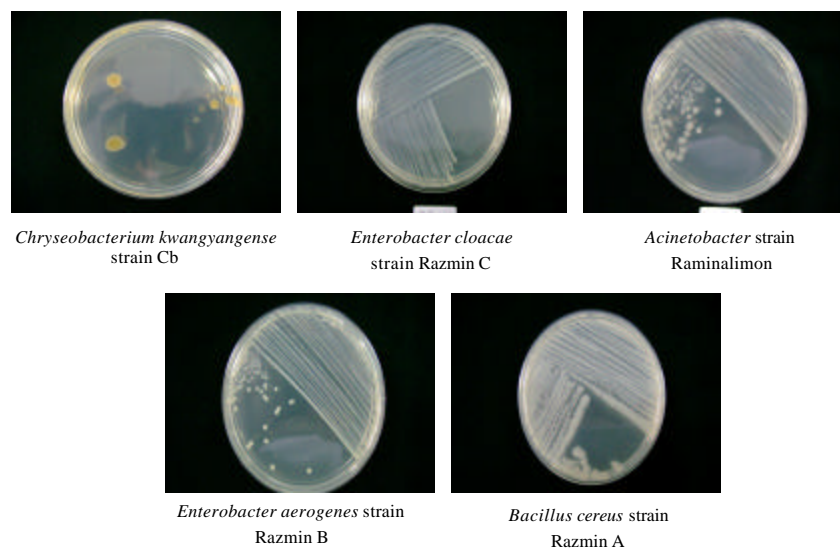


Fig. 1: Bacterial colonies grown on nutrient agar at 39°C overnight

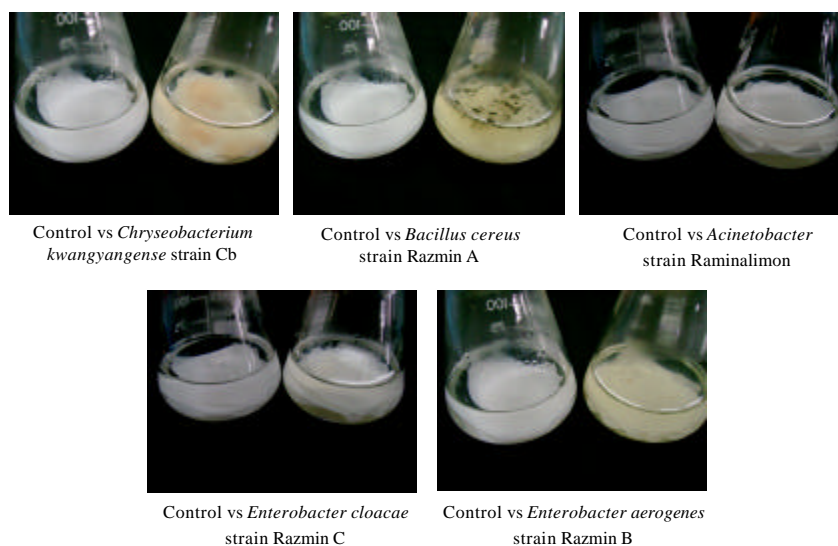


Fig. 2: Bacteria growth and maceration of filter paper after 30 days incubation at 39°C

Konig (2006) had grouped bacteria from termites gut based on their lignocellulolytic activity into two, i.e., hydrolytic and fermentative groups. In this study, all bacterial strains showed both activities. The bacterial strains were able to digest the filter paper as well used the products for growth. Previous studies by Borji *et al.* (2003) also reported that *Enterobacter* and *Acinetobacter* species showed cellulolytic activity. Dugas *et al.* (2001) had isolated and identified a strain of *Chryseobacterium* from the gut of the American cockroach which was fed with a high-fiber diet. The bacterium also produced a yellow pigment colony, similar to the present observation. In this study, it was observed that all five bacterial species isolated from *Coptotermes curvignathus* had the ability to grow by digesting the filter paper supplied in the medium.

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