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Insect gut microbiome - An unexploited reserve for biotechnological application

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PEER REVIEW

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Comments

This work is interesting and contains novel data collection. Situated as a systematic review, this work can be a good data collection for further referencing in metagenomics that is relating to insect microbiology.
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ABSTRACT

Metagenomics research has been developed over the past decade to elucidate the genomes of the uncultured microorganisms with an aim of understanding microbial ecology. On the other hand, it has also been provoked by the increasing biotechnological demands for novel enzymes, antibiotic and signal mimics. The gut microbiota of insects plays crucial roles in the growth, development and environmental adaptation to the host insects. Very recently, the insect microbiota and their genomes (microbiome), isolated from insects were recognized as a major genetic resources for bio-processing industry. Consequently, the exploitation of insect gut microbiome using metagenomic approaches will enable us to find novel biocatalysts and to develop innovative strategies for identifying smart molecules for biotechnological applications. In this review, we discuss the critical footstep in extraction and purification of metagenomic DNA from insect gut, construction of metagenomic libraries and screening procedure for novel gene identification. Recent innovations and potential applications in bioprocess industries are highlighted.

KEYWORDS

Microbiome, Metagenomics, Non-cultivable microbes, 16S rRNA, Gypsy moth, Termite gut

1. Introduction

Insects are the most successful group of animals, both in terms of diversity and survivability in various ecological niches. The insect gut is estimated to contain 10 times more microbes than total cells of the insect and 100 folds more microbial genes than animal genes[1]. Microorganisms colonize the insect gut through food and plays a significant role in digestion and metabolism. While most of the gut microbes are commensals or parasites, some of them are known to play beneficial role for their hosts. Few insect gut symbionts are vertically transmitted and their association is mutually essential such as *Buchnera* sp. in aphid flies[2]. However, such extra cellular associations are thought to be vulnerable to invasion and replacement by transient microbes. Most of the studies were focused on

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understanding the interactions between host and symbiotic microbiota.

Recently, both basic and applied research in biotechnology is focused on the identification of novel genes and proteins/enzymes from various natural sources. Soil and other environmental niches were considered to be the prominent sources of novel biomolecules. Metagenomic approaches allow us to access the genomes of all microorganisms referred as the microbiome. Metagenomics makes it possible to relate potential function of the specific microorganisms within the gut communities. This review describes insect gut metagenomic methodologies, approaches in novel protein/enzyme discovery and their potential industrial applications.

2. Mining the gut microbiome

Experimental insects are washed in suitable sterile buffer and dissected to obtain the complete gut. The gut may be separated into three parts (foregut, midgut and hindgut) (Figure 1). Each part is suspended in extraction buffer and the metagenomic DNA extraction is performed. Cell lysis is a critical step in metagenomic DNA extraction. The enzymatic lysis is gentle and therefore used to lyse the insect gut cells. To access the enriched gut microbiome, the remaining intact microbial cells are washed, lysed again and subsequently DNA was extracted. The mechanical lysis methods such as thermal shocks, homogenization and bead beating can be used to attain complete lysis[3].

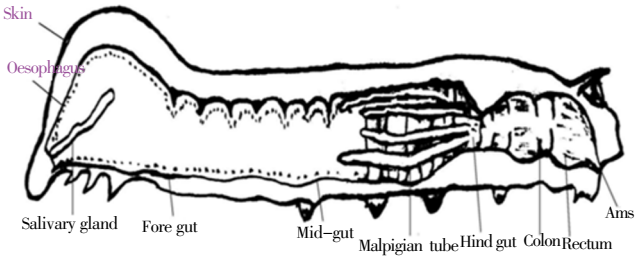

Figure 1. Image of the morphology and different regions of the insect gut.

3. Accessing genomes from cultivable microbes

The gut samples are suspended in phosphate buffered saline, serially diluted and plated on suitable nutrient media. Plates are incubated in a growth chamber at 28 °C for 48 h, and bacterial colonies are categorized based on morphology from the plates with three least countable dilutions. Pure cultures of bacterial isolates are subjected to screening for various enzymes. Subsequently, the DNA from cultures showing important enzyme activities is extracted and the genes coding for the enzymes are cloned and sequenced[4].

4. Accessing genomes of total microbiota

Till date, no specific method has been published, which is universally accepted for the isolation of metagenomic DNA from the insect gut. The major goal of the metagenomic DNA isolation should be to get unbiased access of all microbial communities. In addition, degradation and contamination of the isolated metagenomic DNA should be considered. During metagenomic DNA isolation, shearing or DNA damage should be taken care to obtain the high molecular weight DNA, so that it can be used for construction of metagenomic DNA library using BAC vectors. The metagenomic DNA must be free from other macromolecules without affecting the downstream application such as restriction digestion, PCR and cloning[5].

Most of the gut metagenomic DNA extraction procedure has been adopted from soil DNA isolation methods with slight modifications. In metagenomic DNA isolation, two major strategies have been employed; they are the cell recovery method and the direct lysis method[6].The cell recovery method isolates intact microorganism from the gut content prior to cell lysis, and the cell isolation is carried out either by frequent homogenization and differential centrifugation or by gradient centrifugation in media such as percoll or sucrose[7,8]. Some commercial kits are also available for the isolation of metagenomic DNA from uncultured organisms. However, the isolation protocol must be standardized because most of these kits are not designed for insect gut metagenomic DNA isolation.

In general, individual gut or pooled guts from 10 or 30 larvae or adult insects are placed in 1.5 mL microfuge tubes containing 50 μL PBS and maintained at 4 °C until DNA extraction. The tubes are gently mixed and centrifuged at low speed to pellet the insect gut and DNA is extracted from the bacteria in the supernatant. Cell lysis should be aimed to lyse microorganisms but not the insect gut tissues. Few reports are available for the selective isolation of microbes from soil and from plant tissues. A Nycodenz density gradient was successfully used to separate bacterial cells from soil particles[9]. Similarly, density gradient centrifugation has been applied to enrich microorganisms associated with plant tissues[10]. However, no reports are available for the selective lysis of microorganisms from the insect gut. Since the insect cells are considerably higher than the bacterial cells, the gut suspensions may be subjected to filtration with different pore sized filters. For example, if the suspension is passed through a 1 μm filter, insect cells will be in the retentant and the bacterial cells will be in the filtrate. Subsequently, the bacterial cells can be concentrated through a 0.2 μm filter.

5. Strategies for specific gene enrichment

During the hunt of signified genes, various strategies of gene enrichment were employed which inturn increased the efficiency of cloning prospect and also rushed the exploration of uncharacterized/unknown genes from a reservoir. A typical mode of gene enrichment can be achieved through exposing the microbes under selective pressure such as nutritional selective conditions. Those selective community microbes with preferred phenotype will yield a high responsive/boosted gene enrichment in the particular substrate of interest. The enrichment techniques include suppressive subtractive hybridization phage display and affinity capture[11,12].