Chapter 9

Isolation and Identification of Culturable Endosymbiotic Bacteria from Insects

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Abstract

Insects are the most dominant and successful group in the animal kingdom. This may be due to their highly active defense system, which protects them from various biotic and abiotic factors. Besides these, most insects maintain mutualistic relationships with certain microbes that reside in their bodies, known as endosymbionts. These microbes play vital roles ranging from nutritional supplementation to defense against both abiotic and biotic factors. Endosymbionts are primarily categorized into two types: primary and secondary. Primary endosymbionts are vertically transmitted from mother to offspring, and their roles are considered critical for the nutrition, survival, and development of the host. Most of the obligatory symbionts cannot be cultured on artificial media. Even culturing or isolating the culturable endosymbionts presents a significant challenge. The technique proposed in this protocol for isolating insect endosymbionts involves using specific media and a sandwich method to provide the essential environment required for their growth.

Key words Endosymbionts, Symbiosis, Nutrition, Reproduction, Resistance

1 Introduction

The term symbiosis was coined by Anton de Bary in 1879, referring to the presence of one organism within another without causing harm [1]. Such associations have been studied across the kingdoms of life, with many organisms identified as a major force driving the evolution of life [2–4]. Mereschkowsky (1910) proposed that the evolution of the eukaryotic cell originated from the prokaryotic cells [5]. The roles of endosymbiotic microbes have been recognized in various insects, ranging from reproductive modulation and essential amino acid production to contributing to eukaryotic evolution and pesticide resistance [3, 6–8]. Moran et al. (2008) reported that *Phytosuccivorous hemipteran* insects essentially rely on intracellular symbiotic microbes to fulfill their amino acid requirements [9]. Recently, Jing et al. (2020) demonstrated that *Cryptorhynchus lapathi* harbors dominant gut microbes essential for

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