

ABSTRACT

Soil contains rich in microbial diversity that mainly drives various natural processes and functions. Attempts have been made to study the microbial diversity of soils in many environments to understand the underlying mechanisms and to hunt for novel antibiotic compounds. However, only a small portion (~1%) of microbial cells can be cultured in common laboratory conditions. Thus, soil metagenomic has been recognized as a method to bypass the culture-dependent method for the mentioned purposes. The metagenomic approach is also a key tool for studying soils from more extreme environments, such as the cave ecosystem. However, there is still a lack of studies using cave soils. Thus this study aimed to screen for novel Cas enzymes from the cave soil of Wang-Pra using a sequence-based metagenomic approach. This study utilizes degenerate primers that were designed based on the conserved region of various Cas protein sequences for the screening of the *Cas* genes (9, 12a, and 13b). Other methods including environmental DNA (eDNA) extraction and purification, screening cloning of DNA fragments, and sequencing were used. The study result showed no novel Cas enzymes were successfully identified from the cave soil. A DNA fragment was obtained from the *Cas9* gene screening however the sequencing result showed that it does not correlate to the Cas9 enzyme. Further improvement may need to be made regarding the degenerate primers used, such as decreasing the primer's degeneracy and selecting a new conserved region to obtain better results.

Keywords: Cave Soil; Sequenced-based; Novel Cas Enzymes; Culture-independent Method