

The characterization of Enterobacteriaceae and Pseudomonadaceae isolated from natural salt licks in Sarawak Borneo

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Abstract

Salt lick sites are popular hot spots for hunting among the rural community because many animals are attracted to the sites for mineral licking. The animals roaming freely within the salt lick sites may contribute to the transmission of bacterial pathogens, especially through faecal contamination of the salt lick environments. This study aimed to isolate, identify and characterise bacteria from water and soil of salt lick sites and to determine if there is evidence of the transmission of bacteria between two salt lick sites located within the vicinity of Semait river in the NCR (native customary rights) land area of Long Selaan village, Upper Baram in Malaysian Borneo. Samples of soil (n = 8) and water (n = 7) from the two salt lick sites were collected and plated on Eosine Methylene Blue Agar (EMBA) and MacConkey agar for the isolation of bacteria. Fifteen bacterial colonies were isolated and identified by Matrix-assisted laser desorption ionization time-of-flight mass spectrophotometry (MALDI-TOF-MS), 16S rRNA and API® 20E kit. The bacterial isolates were subjected to (GTG)₅-PCR and antimicrobial analyses. The antibiotics used were Ampicillin (AMP), Tobramycin (TOB), Imipenem (IPM), Amikacin (AK), Trimethoprim-sulfamethoxazole (SXT), Tetracycline (TE), Chloramphenicol (C), Ceftazidime (CAZ), Nitrofurantoin (F) and Norfloxacin (NOR). The result of this study shows the presence of two families of bacteria in the salt lick samples which were Enterobacteriaceae and Pseudomonadaceae. The dendrogram plotted based on (GTG)₅-PCR shows a close genetic relatedness among the bacteria from water and soil as well as in between and within the two salt lick sites. The resistance index of the bacteria from the salt licks ranged from 0.0 to 0.6. This study suggests the potential risk of antimicrobial-resistant bacteria with diverse transmission pathways associated with the salt licks within the area.

1. Introduction

Salt lick, also known as mineral lick, occurs naturally in many parts of Borneo where much terrestrial wildlife depends on its mineral concentration. The salt deposits in the site usually contain essential mineral elements such as phosphorus, sodium, calcium, iron, zinc, and trace elements which are required for supplementing the diet of the animals (Mahaney *et al.*, 1997; Klaus *et al.*, 1998; Brightsmith and Munoz-Najar, 2004; Voigt *et al.*, 2008; Sim *et al.*, 2020). The mineral

composition and concentration of salt licks have been reported to be varied depending on sites (Ayotte *et al.*, 2006). A study conducted on salt lick composition in several salt lick sites in Malaysian Borneo reported that the mineral range of Na was from 0 to 136 mg/kg, K from 454 to 1834 mg/kg, Ca from 0 to 1017 mg/kg and Mg from 450 to 3627 mg/kg (Sim *et al.*, 2020).

Generally, salt lick can be categorized into two types which are the artificial salt lick and the naturally occurring salt lick. Artificial salt lick is normally in the

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