

Molecular detection of new *Bacillus* strains from soil samples of free grazing areas in Basrah province, Southern Iraq

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Abstract: Bacterial contamination is an important indicator for soil quality that could be related to the plant's suitability for animals 'grazing and ultimately human public health. Consequently, the microbial characteristics of soil could be linked to animals' products quality, such as milk and meat. *Bacillus* species is one of the most distributed soil contaminating bacteria and is linked to animals' products quality. Three different grazing areas (Abo Al-Kasib, Al-Seba, and Al-Fawdistricts) in Basrah province were selected to determine the bacterial (*Bacillus*) distribution in soil. Samples were randomly collected from each field of the study. Based on the bacterial cell shape and staining (microscopy), colony morphology, biochemical tests, and the 16S rRNA genes amplification results, the bacterial isolates were identified to be from the genus *Bacillus*, of which *Bacillus cereus* was dominant in all samples. Five new isolates were identified based on 16S rRNA nucleotide sequences. Phylogenetic analysis showed a close relation between three isolates to *Bacillus cereus* with high 16S rRNA gene sequences similarity 99% (SAMU1), 99% (SAMU2) and 98.9% (SAMU3). Furthermore, two isolates showed sequences similarity to *Bacillus paramyoides* (99%, SAMU4) and *Bacillus safensis* (99%, SAMU5). All the above isolates were registered in the NCBI centre under the following names *Bacillus cereus* strain SAMU1, *Bacillus cereus* strain SAMU2, *Bacillus cereus* strain SAMU3, *Bacillus paramyoides* strain SAMU4 and *Bacillus safensis* strain SAMU5 and were assigned the following accession numbers MK418732, MK490900, MK490901, MK490902, and MK490903 respectively. In addition, some isolates were found to be 100% similar to the already identified *Bacillus* strains such as *Bacillus safensis*, *B. pumilus*, *B. paramyoides* and *B. safensis*. The current study investigated and mapped the distribution of *Bacillus* Spp in animals grazing areas that had been subjected to salinity increment.

Keywords: *Bacillus*, *Bacillus cereus*, Grazing field, Soil

How to cite this article: Al-Hejjaj MY, Al-Amara SS, et al (2020): Molecular detection of new *bacillus* strains from soil samples of free grazing areas in Basrah province, Southern Iraq, *Ann Trop Med & Public Health*; 23(S11): SP231147. DOI: <http://doi.org/10.36295/ASRO.2020.231148>

1. Introduction

The soil is considered as one of the richest bacterial environments for isolation of *bacillus* spp. *Bacillus* genus was described for the first time by Cohnin 1872 [1]. This discovery was followed by describing several hundred novel species. *Bacillus* species are rod-shaped, Gram-positive, motile, endospore-forming, and chemoheterotrophic bacteria. They are catalase positive and facultative anaerobic or aerobic [2]. Most of *Bacillus* genus members are characterized by the high level of physiological capabilities and spore formation, which help them to grow in wide range of environments [3]. Some *Bacillus* species members are considered as an important soil contamination factors in many countries [4-6]. Among all *Bacillus* species, *Bacillus cereus* was found to be a distinct family member that plays a big role in soil contamination [7,8]. It is believed that this microorganism contaminates animal products such as milk at unhygienic milking procedure, and meat during slaughter which decreases the shelf life of these products [9]. Furthermore, it is frequently found in vegetables and can be found in food [10,11]. Strains of this genus are able to produce specific toxins that lead to two types of food poisoning with common symptoms either vomiting or diarrhea [9,12,13]. The current study aimed to determine the soil bacterial pollution and to identify *Bacillus* species in soil samples related to free grazing areas, which has a direct relationship to the animal's products hygiene and ultimately human health.

2. Material and Methods

2.1 Sample collection