

Isolation and molecular characterization of potential paraffin wax- degrading thermophilic bacterial strains

(Received: 15. 12. 2014; Accepted: 08. 03.2015)

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ABSTRACT

Three thermophilic bacterial strains that could degrade paraffin wax were isolated from paraffin wax-contaminated soil using enrichment culture and minimal salts medium (MSM) containing n-hexadecane, as reprehensive of paraffin wax, and incubation at 55 °C. When the three isolates, TUSB-B001, TUSB-B002 and TUSB-B003 were tested for their capability for growth and n-hexadecane degradation, they revealed a varying growth patterns and biodegradation efficiency with the isolate TUSB-B001 being the superior. It exhibited a specific growth rate in n-hexadecane-supplemented MSM of 0.11 h^{-1} and growth yield of $1.12\text{ g}_{\text{cells}} / \text{g}_{\text{-hexadecane}}$ compared to 0.065 , 0.883 and 0.055 h^{-1} , $0.74\text{ g}_{\text{cells}} / \text{g}_{\text{-hexadecane}}$ for TUSB-BOO2 and TUSB-BOO3 isolates, respectively. In addition, an efficient paraffin wax degradation of up to 94% was also recorded for the bacterial isolate TUSB-BOO1 compared to 63.8 and 55.2 % for the other ones, respectively. Characterization of bacterial isolates was performed at protein level using polyacrylamide gel electrophoresis (PAGE) and at the molecular level using random amplified polymorphic DNA (RAPD) analysis. Based on the morphological, physiological, biochemical characteristics, and API profile; the isolates were identified as *Bacillus* spp. Moreover, 16S-rDNA gene sequencing confirmed the identification of the strain TUSB-BOO1 at the molecular level as *Bacillus thuringiensis*. The coding gene of the key hydrocarbon degrading enzyme, catechol 2, 3 dioxygenase was detected in the genome of the strain TUSB-BOO1 using PCR amplification and sequencing.

Kew words: Oil contaminated soil, paraffin wax -degrading bacteria, *Bacillus* spp, 16S- rRNA.

INTRODUCTION

One of the most severe problems at any oil field is the wax content due to asphaltene-paraffin-wax deposition. The heavy fraction commonly found in crude oil and known as asphaltene-paraffin-wax precipitate are organic compounds and consist of various forms and combinations of aliphatic hydrocarbons, aromatic hydrocarbons, naphthenes and asphaltine (Magnon *et al.*,

1969). These different forms and combinations give a mixture of natural crystalline precipitate, and stick to the walls of oil producing equipment to decrease the cross section, therefore, asphaltene-paraffin-wax may be deposited throughout the oil production flow lines starting from the oil well reservoirs up to the storage tanks at the refineries.