Isolation, Identification and Characterization of Alkalithermophiles from the Hot Springs of Lake Bogoria of the Kenyan Rift Valley

Lily Simasi

A thesis submitted in partial fulfilment for the degree of Master of Science in Biotechnology in the Jomo Kenyatta University of Agriculture and technology

2009

ABSTRACT

Hot springs are aquatic environments with high temperatures. They harbor diverse groups of micro-organisms that have developed mechanisms to thrive at wide temperature ranges, according to their optimal growth requirements. Research on these microbes is motivated by their great biotechnological potential such as production of useful secondary metabolites and enzymes with industrial application. The objectives of this research were to determine the physico-chemical characteristics of the hot springs in Lake Bogoria, isolate, characterize and identify alkalithermophilies from the hot springs of Lake Bogoria and then screen the isolates for the production of useful metabolites. Samples were collected from the hot springs of Lake Bogoria. The physico-chemical characteristics were established in the field. Enrichment and isolation was done on modified Horikoshi media, at pH 9, and 55^{o}C. Isolates were identified using morphological, physiological, biochemical and molecular characterization. Molecular analysis of Polymerase Chain Reaction amplicons with 16S rDNA region, were amplified using primers specific for bacteria. Restriction Fragment Length Polymorphism (RFLP), sequencing and phylogenetic analysis were done, with *Hae* III and *Msp* I and a Phylogenetic tree was drawn. The physico-chemical parameters established were temperature 90^{o}C and pH 8. Thirty six isolates were obtained and they grew at varied NaCl concentration (2-20%), temperatures (24^{o}C-65^{o}C) and pH (5.7-9). They were non-fermentative, xylanolytic, non-cellulolytic, amylolytic, and some lipolytic and proteolytic. Best enzyme activity was at 55^{o}C. Molecular analysis of Polymerase Chain Reaction amplicons with 16S rDNA region, resulted in only three RFLP patterns with *Hae* III and *Msp* I suggesting that the community structure was homogeneous in the sampling areas. Phylogenetic analysis showed that all the isolates belonged to the domain bacteria, phylum *firmicutes*, class *Bacilli*, order *Bacillates*, family *Bacillaceae* and genus

Bacillus. The hot springs of Lake Bogoria, harbor alkalithermophiles which have the potential to produce useful secondary metabolites such as alkali stable enzymes that can form the basis of bioeconomy if fully exploited. Combining morphological, physiological and molecular approaches and by the use of the modified Horikoshi media, novel isolates related to *B. halodurans* were isolated from the hot springs of Lake Bogoria. Based on the results, the isolates recovered are thermotolerant, alkalitolerant and halotolerant. The isolates also have the potential to produce useful secondary metabolites like enzymes