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BIODIVERISTY OF THERMOPHILIC BACTERIA IN EGYPTIAN HOT SPRINGS

By

EMAN KHALED AHMED
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric. Cairo Univ., 2015

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APPROVAL SHEET

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ABSTRACT

Geothermal water samples collected from Dakhla Oasis, Kharga Oasis, Pharaoh Baths and Ras Sedr hot springs in Egypt were explored for the isolation of industrially efficient thermostable amylase-, protease- and lipase- producers. Of 170 enzyme-producing isolates secured from colonies developed on agar media, 12 superior ones were subjected to morphological characteristics and biochemical profiles. Cells appeared cocci and spiral with the majority as bacilli. Adopting the Diagnostics GN/GP 24 (Ref. 1001, 1002), the tested candidates successfully utilized various substrates as carbon and nitrogen sources besides their abilities to produce a number of exoenzymes. Six potent amylase-, protease- and lipase-producing thermophiles, two for either, were further identified by 16S rRNA gene sequencing. The amylase-producers were identified as *Aneurinibacillus thermoaerophilus* and *Bacillus licheniformis* with respective similarity percentages of 99.48 and 100. Genetic analysis of protease-producers showed the similarity to *B. licheniformis* and *B. sonorensis* (90.02 and 98.14 % identities). Both lipase-producing isolates described as *B. licheniformis* with similarity percentages of 98.80 and 100. The Plackett-Burman Multifactorial Design was implemented to screen the limiting components for growth and subsequent use of Central Composite Design to tailor a suitable medium that supports exponential growth and consequently the enzyme production of the tested thermophilic bacteria. For optimization of protease production by *Bacillus licheniformis* (isolate DO24), the applied Placket-Burman methodology selected eleven effective factors including skim milk, peptone, yeast extract, $CaCl_2$, $MgSO_4.7H_2O$, NaCl, KH_2PO_4, inoculum size, pH, temperature and incubation time. Among those; skim milk, yeast extract, inoculum size and incubation period deemed the most influential factors. Adopting the Central Composite Design, the optimized protease activity was achieved at the respective component records of 40 ml L^{-1}, 4.0 g L^{-1}, 40 ml L^{-1} and 24 h. For amylase of *Aneurinibacillus thermoaerophilus* (RS10); yeast extract, KH_2PO_4 and incubation period were the most supportive. Tween 80, peptone, yeast extract, pH and incubation temperature obviously optimized lipase production by *Bacillus licieniformis* (KhO24). This study proves that Egyptian hot springs are beneficial reservoirs for thermostable enzyme-producing microbiota of great importance for various bio-industrial applications.

Key words: Hot springs, thermophiles, thermostable enzymes, 16S r RNA gene sequencing, Plackett-Burman Design, Central Composite Design.

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INTRODUCTION

Hot springs, the very special niches, are those produced by the immergence of geothermal heated groundwater from the earth's crust and occur in a few widely separated locations of the world and are observed in the areas that having active volcanoes. Of the popular ones, are Yellowstone national park (USA), Suryakund (Bihar, India), Sohna hot spring (Sohna, Delhi), Atri hot spring (Khordha, Odisha), Manikaran hot spring (Himachal Pradesh, India), Cimanggu hot spring (West Java, Indonesia), hypersaline and heliothermal Ekho Lake (East Antarctica), Garampani hot spring (Assam), Unapdev and Sunapdev hot spring (Maharashtra, India) and Bakerswar hot spring (West Bengal, India) (Ulfah *et al.*, 2011). These particular environments are densely accommodated by a great variety of microorganisms that possessing the capability to withstand the prevailing rigorous conditions. They are developing unique resistance to perform reactions and activities at either acidic or alkaline pH, temperatures falling in the range 45-140 °C, or very close to the freezing point of water, high pressures or in non-aqueous environments and water/solvent mixtures. Such characteristics represent an excellent biotechnological tool to support and catalyze non-limited reactions in severe conditions (Elleuche *et al.*, 2015). Here, it could be realized that thermophiles, in general, are proved a rich source of extremoenzymes and therefore gained an importance in what so

called “white biotechnology” which is defined as the use of several microorganisms and their enzymes in the industrial processes beside the production of certain materials and chemicals (Bergquist *et al*., 2014; Elleuche *et al*., 2014). The whole cells, their macromolecules or metabolites are commonly using in bioremediation, bioenergy, biomining and biosurfactant production (Zerva *et al*., 2019; Kor-Bicakci *et al*., 2020). Among the thermophile cell components, extremoenzymes are occupying a non-tiny place on the map of the biotechnological applications of microorganisms in the various industrial processes. Actually, the thermoenzymes are considered among the pillars of industrial processes referring to the fact that higher temperature are necessary to improve the solubility of many reaction components (mainly polymeric substrates) and minimize the hazards of contamination. Of those enzymes; amylases, cellulases, chitinases, esterases, lipases, pectinases, proteases, pullalanases and xylanases are of special concern. Principally; the industrially important amylases, proteases and lipases account the majority of the total global enzyme sale.

Initially, the term amylase was used originally to designate an extracellular enzyme capable of hydrolyzing α-1, 4-glucocidic linkages in polysaccharides containing three or more glucose units. This enzyme is indispensible in the various biotechnological approaches, cosmetics, nutrition and pharmacy (Kumar and Srikumar, 2013). Amylase produced by thermophiles might be thermo stable, a characteristic that is

necessary for several applications requiring relatively high temperature such as starch industry which involves the processes of gelatinization and liquefaction (Mehta and Satyanarayana, 2016). Dash *et al*. (2015) reported that, the *Bacillus* species; *B. amyloliquefaciens, B. licheniformis, B. stearothermophilus* and *B. subtilis* are having the ability to synthesize the α-amylase enzyme. The results of 16 S rRNA sequencing of Ardhi *et al*. (2020) indicated that the amylase-producing LBKURCC190 isolates had the highest similarity (> 98 %) with *Bacillus* spp.

Proteases, as some of the cornerstone exoenzymes unavoidable for non-limited industries, hydrolyze the peptide bonds present in proteins and polypeptides. They have a wide biotechnological applications such as leather, pharmaceutical and food industries as well as manufacture of protein hydrolyzate and waste processing industry. Abdollahi *et al.* (2020) isolated 36 thermophilic bacteria from Gavmesh Gli hot spring in Sareyn, North West of Iran. All the secured isolates were potentially protease producers, among those five were characterized by high enzyme activities. Morphological, biochemical and molecular analyses adopting the 16 S rRNA gene sequencing indicated that four isolates (DH15, DH16, DH20 and DH29) were assigned as *Thermomonas hydrothermalis* while one (PA10) was identified as *Bacillus altitudinis*.

Lipases, as well, are universally applied in a great number of industries; they are defined as triacylglycerol acylhydrolases