Research Article

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Hydrolytic Enzyme Production by Thermophilic Bacteria Isolated from Saudi Hot Springs

https://doi.org/10.1515/biol-2018-0056
Received May 1, 2018; accepted September 29, 2018

Abstract: Hydrolytic enzyme production by thermophilic bacteria isolated from hot springs in the southern region of Saudi Arabia was investigated. The physical and chemical properties of the hot springs prove to be an important environment for hydrolytic-enzyme-producing thermophilic bacteria. Eighty-four bacterial isolates were obtained from three hot springs: Al-Majardah, Al-Khubah and Al-Ardah. Screening of the isolates for enzyme production indicated that 78 isolates showed activity for one or more enzymes. Molecular identification and phylogenic analysis of selected promising isolates confirmed the identity of the isolates as *Bacillus aerius, Bacillus licheniformis* and *Bacillus sonorensis*, which have potential to produce the target enzymes α-amylase, protease and lipase, respectively. Optimization of hydrolytic enzyme production by bacterial strains was investigated using kitchen waste as a cheap carbon energy source. Maximum enzyme production was achieved after 72 hours of incubation at the beginning of the stationary phase of growth. Enzyme production was dependent on the initial pH value in the range of pH 7.5-8.5 and an optimal incubation temperature of between 55-60°C. Enzyme production increased gradually in proportion to the kitchen waste concentration; whereas maximum lipase production was gained at 5.0% (w/v) kitchen waste, 7.0% (w/v) of waste was optimal for both α-amylase and protease productivity. The results indicated that hot springs in Saudi Arabia are a substantial source of thermophilic bacteria producing industrially important enzymes using cheap and unexploited waste.

Keywords: Hot springs, Thermophilic bacteria, Enzymes, Production, Kitchen waste

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1 Introduction

Hot springs are amongst the hottest places in the world, and these regions are the main habitats of thermophilic bacteria [1]. These springs have various chemical and physical properties that support the growth of thermophilic bacteria [2]. There is a gap in knowledge regarding the potential of hot springs as a resource in Saudi Arabia [3]. There are ten hot springs throughout Saudi Arabia; however, the microorganisms in these springs have yet to be investigated and characterized [4]. Most geothermal activity is located in the Gazan and Al-lith regions and is accompanied by temperatures ranging from 50 to 120°C [5]. This untapped resource has potential for heating purposes, tourism, therapeutic benefits and as a source of bioactive metabolites. In general, few reports have been published concerning biological activities in hot springs in Saudi Arabia [6]. Thermophiles are organisms that have evolved to grow in extreme environments and grow best at temperatures between 45 and 80°C. Due to their harsh environment, thermophilic bacteria are a reservoir for biodiversity, molecular phylogeny, and the production of unique industrially useful enzymes and other compounds [7]. The major challenge for thermophilic bacteria is their survival and the production of thermo-stable enzymes and other bioactive molecules at high temperatures. Microbial enzymes occupy a prominent position in modern biotechnology. Bacterial α-amylase, protease and lipase enzymes are used extensively in biotechnology processes [8]. One of the appealing characteristics of these enzymes is their thermostability, which makes them extremely interesting for industrial purposes [9]. Global market growth and continuing industrial demand has led to the

extensive production and use of thermo-stable enzymes [10]. The enzymes α-amylase, protease and lipase are the major examples of enzymes found in the majority of thermophiles [1, 8]. According to the Food and Agricultural Organization, one-third of food produced globally for human consumption is wasted [11]. The situation in Saudi Arabia is even more critical and complex [12]. In recent years, interest has focused on biochemical production of beneficial products, such as enzymes, from kitchen waste [13]. Therefore, the current investigation aims to isolate thermophilic bacteria capable of producing industrial enzymes using kitchen waste as a cheap and unexploited carbon source.

2 Methods

2.1 Physico-chemical properties of the hot spring samples

The three hot springs chosen for this study; Al-Khubah, Al-Ardah and Al-Majardah are located in the southern region of Saudi Arabia. The water samples were collected in 500 ml glass bottles. The temperature, pH, electrical conductivity (EC) and total dissolved solid (TDS) of hot spring water samples were measured in real time during sampling using Benchtop Meters (OAKTON). The heavy metal content was analysed by Inductive Coupled Plasma-Optical Emission Spectrometry (TCP-OES) [14]. Na^+, K^+, Ca^{2+} and Mg^{2+} levels were determined using Energy Dispersive X-ray Fluorescence Spectrometer (ED-XRF) [15]. Phosphate concentration was determined by the stannous chloride method [16] and nitrate concentration was measured by the ultraviolet spectrophotometer screening method [17]. Chemical analysis of other components was performed in the laboratory using standard techniques [18].

2.2 Isolation of thermophilic bacteria

Two different isolation media were used to isolate the thermophilic bacteria, Thermus agar medium [19] and ATCC medium 697 [7]. Thermus agar medium contained (milligram /litre); nitrilotriacetic acid (100), $CaSO_4$ (60), $MgSO_4$ (100), KNO_3 (103), $NaNO_3$ (689), Na_2HPO_4 (111), $MnSO_4$ (2.2), $ZnSO_4$ (0.5), $CuSO_4$ (0.016), H_3BO_3 (1.0), Na_2MoO_4 (0.025), $CoCl_2$ (0. 046), $FeCl_3$ (0.28), tryptophan (1.0), yeast extract (1000) and twenty grams of agar. The second isolation medium, ATCC medium 697, contained (%); NaCl (0.5), yeast extract (0.2), beef extract (0.4), peptone (0.5), and agar (2). All media were adjusted to pH 7.5 prior to autoclaving at 121°C for 20 min at 1 kg/cm^2. Two isolation methods were employed, 100 ml aliquot of each water sample was filtered using membrane filters (0.22 µm Millipore Corporation, Bedford), and the filters were then transferred onto the agar plates. The plates were incubated for 48 h at 55°C [20]. To enrich the thermophilic bacteria's ability to produce α-amylase, protease and lipase enzymes, 5 grams each of sterile starch, casein and tributyrin were added separately to hot spring water samples, mixed and incubated for 2 weeks at 55°C [21]. The 100 µl of the diluted samples was transferred onto agar plates and incubated at 55°C for 48 h.

2.3 Screening of thermophilic bacterial isolates for hydrolytic enzyme production

Bacterial isolates were screened using hydrolytic plate assay tests on starch, casein and tributyrin agar plates for testing α-amylase, protease and lipase production, respectively. After incubation at 55°C for 48 h, starch hydrolysis was determined by flooding the plates with iodine solution [22]. Following treatment with hydrochloric acid on the casein agar plates, the isolates showing zones of clearance were selected as protease producing bacteria [23]. Lipid hydrolysis of the isolates was screened using tributyrin agar medium containing phenol red; a colour change from pink to yellow around the colonies indicating lipase production [24]. For qualitative screening of enzymes, enzyme index was measured [25]. The promising isolates showing a high potency index were then selected for further study.

2.4 Identification of the promising isolates

Bacterial genomic DNA of promising isolates was extracted from 5 ml bacterial cultures grown overnight in nutrient broth using a modified QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA) [26]. The extracted DNA from each bacterial isolate was used as a template for amplification of the 16S rRNA gene using the universal primers 5' CCA GCA GCC GCG GTA ATA CG 3' and 5 'ATC GG(C/T) TAC CTT GTT ACG ACT TC 3'. PCR reactions were carried out in a 50 µl volume containing 10 mM of Tris-HCl (pH 8.3), 50 mM of KCl, 2.5 mM of $MgCl_2$, each dNTP at a concentration of 0.2 mM, 1.25 IU of Taq polymerase, each primer at a concentration of 0.2 µM, and 1 µl of the DNA template. The volume was made up to 50 µl with water. PCR was performed according to the following programme: 10 min denaturation at 94°C, followed by 35 cycles of 1 min denaturation at 94°C, 1 min

annealing at 55°C, 2 min extension at 72°C, and a final extension step of 10 min at 72°C. Then, 5 µl of the amplified mixture was analysed using 1.5% agarose gel dissolved in 0.5X of TBE buffer and containing ethidium bromide. The gel was run on an electrophoresis unit for 30 min at 150 V, for gene product migration and separation. The migrated bands were observed under UV light and photographed using a gel documentation system. To verify the presence of appropriate sized amplicons, the PCR product for each isolate was compared with a 1 kb DNA ladder. A product of the correct size was purified with a Taka R Agarose Gel DNA purification Kit Ver. 2.0 and sequenced in both directions using an ABI 3730 automated sequencer (Macrogen, Korea). The sequences of the selected isolates were aligned and compared with the deposited sequences in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To determine the taxonomic position of the isolates, a phylogenetic tree was constructed with MEGA version 5.0 program using a neighbour-joining algorithm. The Jukes-Cantor distance estimation method with bootstrap analyses for 1000 replicates was also performed. The nucleotide sequences of the amplified 16S rRNA genes of the selected strains reported in this study have been deposited in the GenBank nucleotide sequence databases under different accession numbers.

2.5 Production of Hydrolytic enzymes

Kitchen waste (KW) used as the carbon source for hydrolytic enzymes production was collected from King Khalid University restaurants and stored at 4°C, following drying, crushing and grinding, it was analysed for protein, fat and carbohydrate contents [27]. The selected isolates were further screened for the production of hydrolytic enzymes (α-amylase, protease and lipase) by submerged fermentation. The inoculum was prepared by growing a bacterial culture in nutrient broth medium at 55°C/150 rpm for 24 h. Erlenmeyer flasks (250 ml) containing 50 ml of ATCC medium 697 supplemented with 1% KW as the sole carbon source. The medium was inoculated with 1% inoculum (approximately $2x10^6$ CFU ml^{-1}) and incubated at 55°C in a shaking incubator at 150 rpm for 96 h. Samples were harvested at 12 h intervals, and growth was determined through the measurement of optical density (OD_{600} nm). The cells were removed by centrifugation at 6000 rpm for 20 min, and the obtained supernatant was then used for enzymes assay [28]. The factors affecting enzymes production such as incubation period (12- 96h), pH (6-9), temperature (45-70°C) and kitchen waste concentration (1-9%) were investigated.

2.6 Enzyme assays

2.6.1 α- amylase activity

The α-amylase activity was determined by using Bernfeld method [29]. A 1 ml amylase solution and 2 ml 0.5% starch solution in 0.1 M Tris– HCl buffer (pH 7.4) were incubated for 30 min at 50°C. The reaction was stopped by addition of 2 ml DNS, retained in boiling water for 5 min and then diluted by addition of 10 ml distilled water. The absorbance was measured using a double beam UV/Vis scanning spectrophotometer (Model: Shimadzu, 1601PC) at 489 nm. One unit of enzymatic activity (U) was defined as the amount of enzyme that produced 1 µmol of glucose per minute under the assay conditions. All experiments were performed in triplicate.

2.6.2 Protease activity

Protease activity was determined by applying a modified method previously used by Takami et al. [30]. The mixture containing 0.5 ml of 1% casein in 0.025 Tris-HCl buffer (pH 7.4) was incubated with 0.5 ml of enzyme solution at 50°C for 10 min. The reaction was stopped by adding 0.5 ml of 0.4 M TCA. The mixture was filtered and 0.5 ml of the filtrate was mixed with 2.5 ml of 0.4 M Na_2CO_3 plus 0.25 ml of Folin Ciocalteu's reagent and incubated for 30 min at room temperature. The absorbance of the solutions was read against the blank sample at 660 nm using a double beam UV/VIS scanning spectrophotometer (Model: Shimadzu, 1601PC). One unit (U) of protease activity was defined as the amount of enzyme that produce 1 µmol tyrosine per min under assay conditions.

2.6.3 Lipase activity

Lipase activity was determined by the methods described by Abd-Elhakeem et al [31]. The reaction mixture containing 0.1 ml lipase solution, 2.4 ml phenyl acetate (165 µM) in Tris HCl buffer (0.1 M) at pH 7 and 1% (v/v) Triton X-100 were incubated at 50°C for 10 min. The liberated phenol was determined by Folin Ciocalteu reagent and the absorbance was measured at 750 nm. One unit of lipase activity (U) was defined as the release of 1 µmol of phenol per min under the optimum conditions. All experiments were performed in triplicate.

2.7 Statistical analysis

Analysis of variance using one-way ANOVA was carried out. Significance was determined as $P \leq 0.05$ using Minitab for Windows software package version 15. The error bars represent standard error of the mean for n=3.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

3.1 Physico-chemical properties of water samples

The physical and chemical properties of water samples obtained from hot springs were examined to evaluate their effect on the isolation of thermophilic bacteria (Table 1). The results showed that Jazan hot springs have a temperature gradient ranging from 60 to 70°C, while Al-Majardah hot spring has a temperature of 55°C. All of these springs had a neutral or slightly alkaline pH; the lowest value was 7.2 in the Al-Ardah site and the highest was 8.2 in the Al-Majardah site. Conductivity and TDS also varied, the highest ratio of which was observed for Al-Majardah hot spring reaching 5100 µS cm^{-1} and 3147 mg l^{-1}, respectively. The results of heavy metals analysis indicated that all samples were free of toxic elements, where Cd, Cr, Fe, Co and Ni were not detected, while Cu, Pb, Zn, and Mn were detected but in very low concentrations. The remaining elements and compounds including Na, Ca, K, nitrate, ammonium, phosphate, and sulphate were variable among the hot springs (Table 1). The sodium, calcium and potassium concentrations in the Al-Majardah spring (14.64%, 12.14% and 32.95%, respectively) were high compared to those in the Al-Khubah and Al-Ardah hot springs. The concentrations of NH_4^+, NO_3^-, SO_4^{2-}, and PO_4^{3-} nutrients in Jazan springs were lower than those of the Al-Majardah spring.

Table 1. Physic-chemical properties of water samples from hot spring sites

Parameters	Hot springs		
	Al-Khubah	Al-Ardah	Al-Majardah
Temperature (°C)	70	60	55
PH	7.7	7.2	8.2
EC (µS cm-1)	4128	4227	5100
Total dissolved solids	2661	2806	3147
Na^+ mgl^{-1}	250	280	321
Ca^{2+} mgl^{-1}	135	140	157
K^+ mgl^{-1}	75	88	117
Mg^{2+} mgl^{-1}	11.011	11.89	13.57
Mn^{2+} mgl^{-1}	0.004	0.003	0.033
Cu^{2+} mgl^{-1}	0.003	0.003	0.006
Cd^{2+} mgl^{-1}	Nil	Nil	Nil
Pb^{2+} mgl^{-1}	0.002	0.001	0.001
Cr^{3+} mgl^{-1}	Nil	Nil	Nil
Fe^{2+} mgl^{-1}	Nil	Nil	Nil
Co^{2+} mgl^{-1}	Nil	Nil	Nil
Ni^{2+} mgl^{-1}	Nil	Nil	Nil
Zn^{2+} mgl^{-1}	0.002	0.003	0.009
NH^{4+} mgl^{-1}	47.1	46.4	55.3
NO^{3-} mgl^{-1}	7.46	7.23	8.41
SO_4^{2-} mgl^{-1}	2300	2040	3000
PO_4^{3-} mgl^{-1}	8.4	8.1	9.7

3.2 Isolation and Screening of hydrolytic-enzymes-producing bacteria from hot spring

A total of eighty-four thermophilic bacterial isolates were isolated from all hot spring sites using two isolation media. The highest numbers of thermophilic bacteria were isolated from the Al-Majardah (33 isolates) and Al-Khubah (30 isolates) hot springs, while fewer isolates were collected from the Al-Ardah hot spring (21 isolates). The occurrence of isolates in different localities may be a result of the prevailing environmental conditions and the chemical properties of the water (Table 1).

All bacterial isolates were screened to evaluate their ability to produce hydrolytic enzymes, α-amylase, protease and lipase using ATCC medium 697 [7]. Preliminary tests on media enriched with the substrate for each enzyme showed that 77 isolates exhibited enzymatic activity for one or more enzymes. Amongst the 84 thermophilic bacterial isolates, 33.33%, 29.76% and 28.57% of the strains possessed high amylolytic, proteolytic and lipolytic activities, respectively. The enzyme index differed, suggesting that some strains displayed higher activity than others. The highest α-amylase activity was produced by isolate KKU-KS5 (3.6 mm) from the Al-Khubah hot spring while, the highest protease and lipase activity were produced by isolates KKU-MS4 (4.33 mm) and KKU-MS14 (6.0 mm) from the Al-Majardah hot spring site (Figure 1). A detailed study of the promising bacterial isolates was then conducted to optimize enzymes production.

3.3 Bacterial identification using 16S rRNA gene sequencing and phylogenetic analysis

Three promising isolates: KKU-KS5, KKU-MS4 and KKU-MS14 were selected for identification according to their production of α-amylase, protease and lipase, respectively. The molecular genetics of the isolates was determined, to ensure accurate identification and establish the correct phylogenetic position. The primers used amplified almost 996 pb of the 16S rRNA target gene. After sequencing, the 16S rRNA gene sequences obtained for the three selected isolates were compared with the deposited sequences in GenBank for each isolate using the BLAST search of the National Center for Biotechnology Information (NCBI) databases. Alignment of the 16S rRNA gene sequences of these isolates, along with sequences obtained using a BLAST search, revealed 99% similarity to different bacterial species. The strains were virtually identical to genus *Bacillus* and 3 different species. These were *Bacillus aerius* (KKU-KS5), *Bacillus licheniformis* (KKU-MS4), and *Bacillus sonorensis* (KKU-MS14) with 99% similarity each. The three genes were deposited in GenBank, and the obtained accession numbers were recorded as KY982904, KY982906 and KY982908. In addition, Figure 2 illustrates and confirms the phylogenetic relationship among the bacterial isolates and similar strains deposited in GenBank.

3.4 Hydrolytic enzymes production

Kitchen waste (KW) collected from university kitchens was used as the sole carbon source for enzymes production. The total amount of carbohydrate, protein and lipid in KW was 76.1%, with the highest proportion being that of carbohydrate (46.2%). The total amounts of protein and fat were 18.7% and 11.2%, respectively. The production of α-amylase, protease and lipase by selected promising bacterial strains, *Bacillus aerius* (KKU-KS5), *Bacillus licheniformis* (KKU-MS4) and *Bacillus sonorensis* (KKU-MS14) using KW as a carbon source was investigated. The factors affecting enzyme production, including incubation period, pH, temperature and kitchen waste concentration were investigated.

3.4.1 Effect of incubation time

The fermentation process was carried out for up to 96 h with measurement of bacterial growth and enzyme

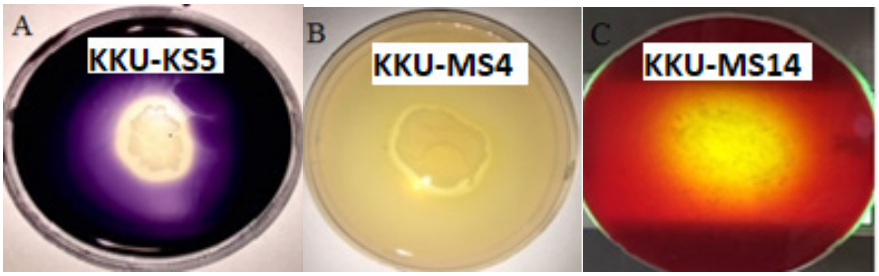

Figure 1. Hydrolysis of (A): Starch, (B): Casein and (C): Tributyrin by selected isolates using ATCC medium 697.

activity at 12 h intervals. The results revealed that significant ($P \leq 0.05$) enzyme production was initiated after the first interval, but maximum production was obtained after 72 h yielded 43.53 Uml^{-1} of α-amylase, 135.2 Uml^{-1} of protease and 121.26 Uml^{-1} of lipase (Figure 3). Further incubation after this optimum period did not increase the enzymes activity, and a steep decrease down to 38.13%, 51.84% and 49.50%, respectively was observed after 96 h of incubation. Maximum enzyme production was obtained at the beginning of the stationary phase of bacterial growth, which suggests that enzyme production and bacterial growth correlate.

3.4.2 Effect of initial pH

The effect of initial pH value on the production of enzymes was investigated. The results showed that enzymes production was dependent on pH value (Figure 4). Maximum α-amylase and lipase production were achieved significantly at pH 7.5 ($P \leq 0.05$), while optimum protease production was obtained at pH 8.5. Enzymes production beyond these optimum pH values was markedly decreased. Enzymes production declined significantly ($P \leq 0.05$) at acidic pH levels, decreasing at pH 6.0 down to 70.93%, 76.18% and 78.28% for α-amylase, protease and lipase, respectively. This suggests that these enzymes are more active in an alkaline pH.

3.4.3 Effect of incubation temperature

To study the effect of temperature on enzyme production, a fermentation process was carried out in the temperature range of 45 to 70°C (Figure 5). The results indicate a significant ($P \leq 0.05$) relationship between enzyme production and incubation temperature up to 55-60°C. However, an increase in the temperature beyond 60°C leads to a decline in enzyme production. The activity of α-amylase and protease was optimal at 60°C with no significant reduction ($P > 0.05$) of α-amylase production at 65°C. Maximum lipase production was recorded at 55°C. Further increases in the incubation temperature beyond 55°C led to a rapid decline in lipase activity.

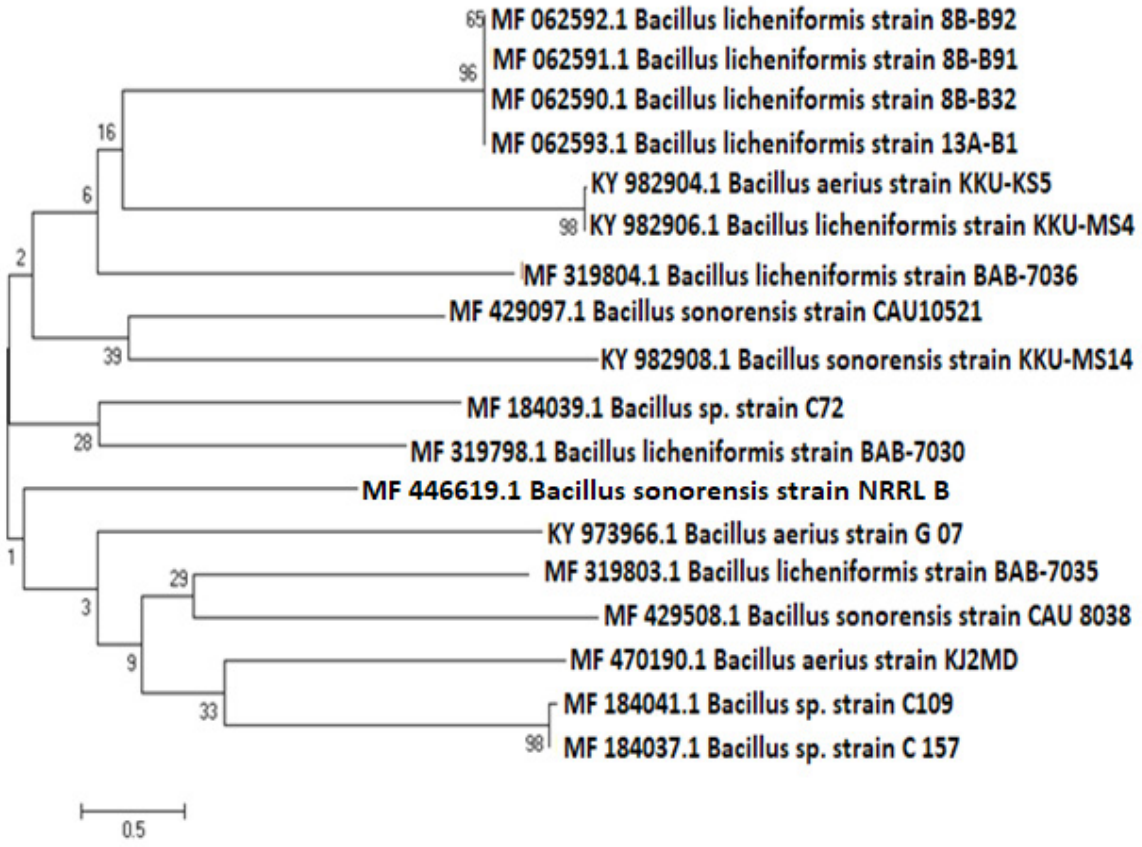

Figure 2. Phylogenetic relationship among the three bacterial isolates and other bacterial strains deposited in GenBank.

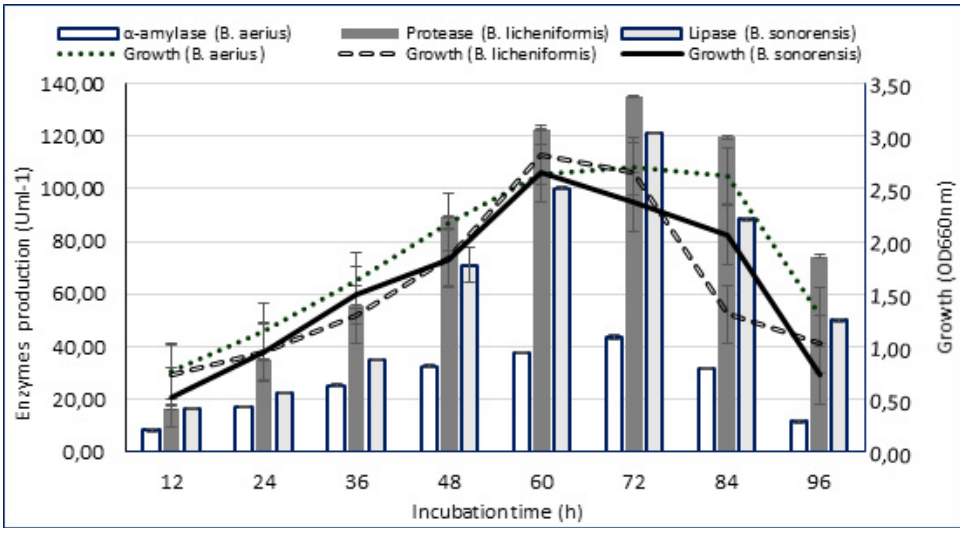

Figure 3. Effect of incubation time on bacterial growth and enzymes production.

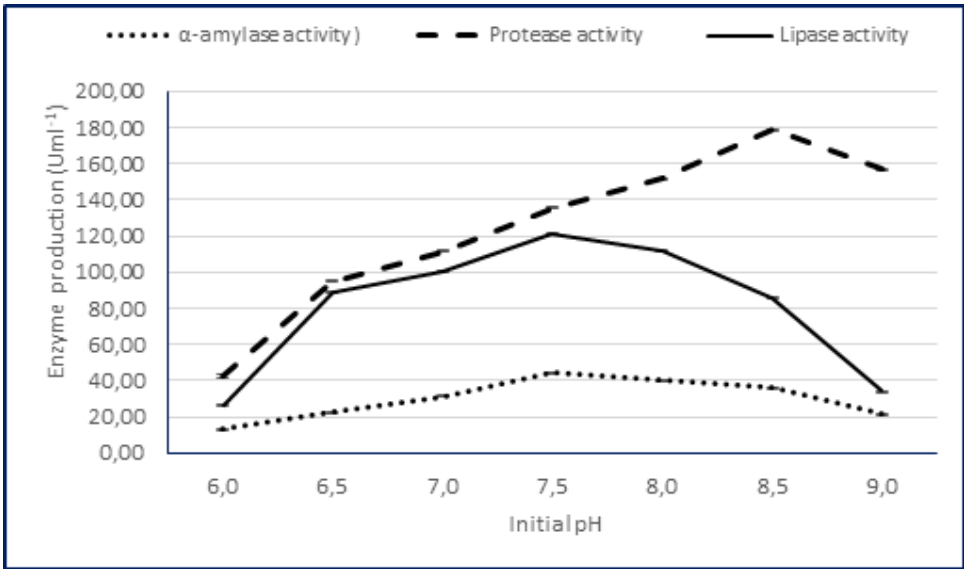

Figure 4. Effect of initial pH on enzymes production.

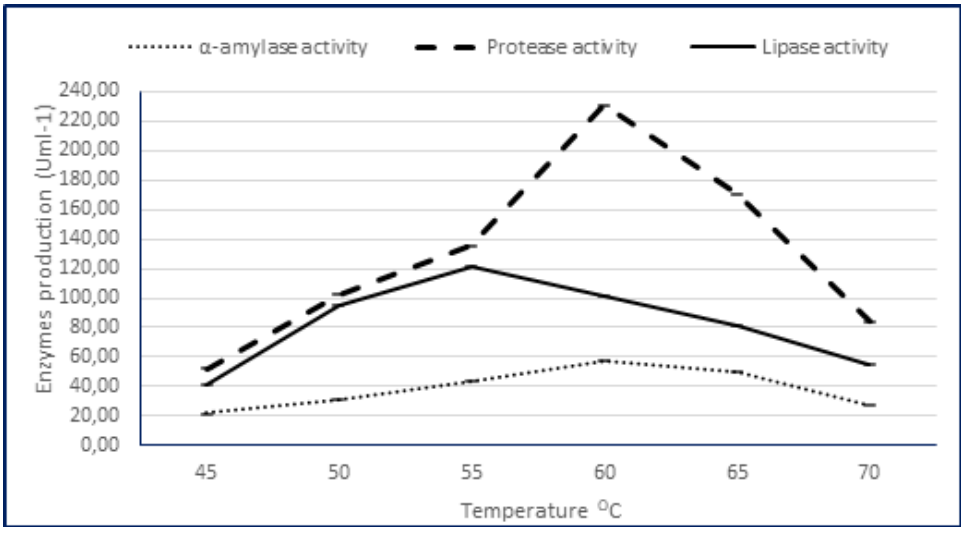

Figure 5. Effect of incubation temperature on enzymes production.

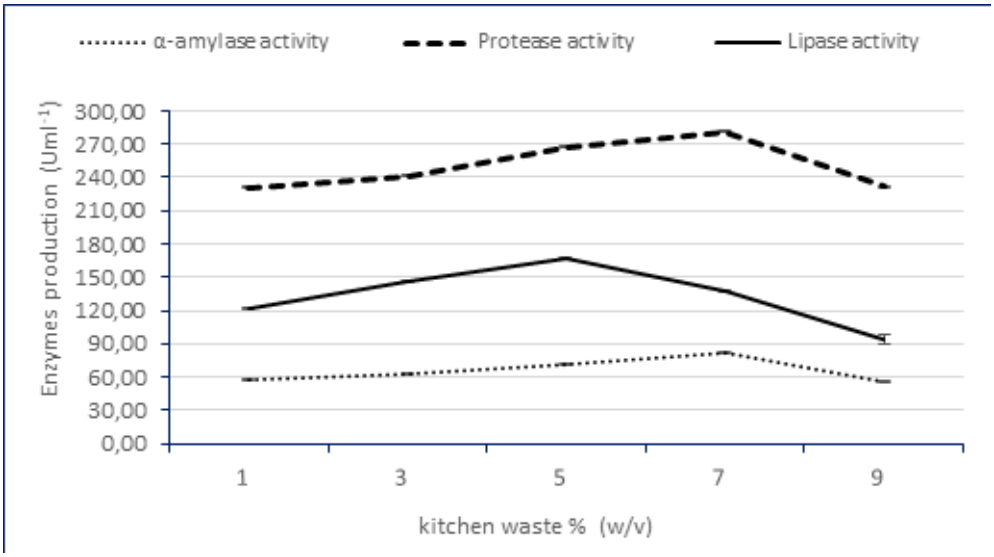

Figure 6. Effect of kitchen waste concentration % (w/v) on enzymes production.

3.4.4 Effect of kitchen waste concentration

Different concentrations of the kitchen waste used as the sole carbon source were examined to establish the level for maximal enzyme production (Figure 6). Enzyme production increased gradually in proportion to the concentration of KW, it was significantly ($P \leq 0.05$) rising to 5.0% (w/v) for lipase and 7.0% (w/v) for both α-amylase and protease. Enzyme production was reached 81.43 Uml^{-1} for α-amylase, 281.36 Uml^{-1} for protease and 167.3 Uml^{-1} for lipase. It is interesting to note that optimal enzyme activity achieved dramatically decreased with a high concentration of KW. An increase in kitchen waste concentration to 9% led to a rapid decline in enzyme production of 31.27%, 17.77% and 33.45% for α-amylase, protease and lipase, respectively.

4 Discussion

The main hot springs are concentrated on the western and southwest coast of the KSA due to the volcanic warming associated with the opening of the Red Sea in this area [4]. The physical and chemical properties of water samples obtained from three hot springs located at the Southern region of Saudi Arabia were examined to evaluate their effect on the isolation of thermophilic bacteria. The pH and conductivity of the hot spring samples were in accord with the results reported by Basahy [32] and Lashin and Al Arifi [33], while the temperature of the Al-Khubah hot spring varied. It was approximately 57°C in 1994, however, it has recently ranged between 70 and 80°C. The change in temperature may be explained by changes in the tectonic features and crust thickness of the area, as these hot springs are located along the west coast region, which recently experienced an earthquake [34]. The pH of hot springs fell within a small range of values (7.2–8.10) which is suitable for growing thermophilic bacteria [35]. The high electrical conductivity (4128 to 5100 μS/cm) may be due to the presence of high mineral concentrations [4]. Indeed, the chemical analysis results were in agreement with previous reports [5, 31]. Additionally, the hot springs in Saudi Arabia have a temperature range from 50-70°C, and therefore have a high chance of supporting the survival of thermophilic bacteria [36].

The current study found that thermophilic bacteria capable of producing enzymes were present in all studied samples. The occurrence of isolates in the different localities may be affected by the prevailing environmental conditions and the chemical properties of water. The high number of bacterial strains isolated from the Al-Majardah hot spring may be due to a greater availability of nutrients (Table 1). The hydrolase-producing thermophilic bacteria were successfully isolated from Saudi hot springs at 55°C and a pH of 7.5 [7, 35]. The pH of the hot springs fell within a small range of values (7.2–8.1) that is suitable for growing thermophilic bacteria [5]. Hydrolytic enzymes have extensive biotechnological applications, each of which requires unique enzyme properties with respect to specificity and thermal stability [37]. Therefore, all thermophilic bacteria isolates were screened to evaluate the hydrolytic activity of starch, casein and tributyrin by producing α-amylase, protease and lipase enzymes, respectively. The environmental conditions and the chemical properties of water samples from the different hot springs may be the reason for the differences in

enzyme production. The findings of Khalil [38] support those of the current study by suggesting that thermophilic bacteria isolated from hot springs in Saudi Arabia were producing high concentrations of lipase and amylase enzymes. The bacterial diversity detected in the current study was in accordance with that of bacteria occurring in the hot springs of Saudi Arabia [35, 39]. Most of these promising thermophilic bacterial isolates belong to genus *Bacillus*. The appearance of *Bacillus* strains in all hot springs that have been studied may be explained by the extensive degree of bacterial transport occurring across regions and continents, which adapt the *Bacillus* spores to environmental stress and meet their simple nutritional needs for growth [40]. The limited variety of bacterial species detected in Saudi Arabia hot springs may be due to high temperature and low organic nutrients [41]. Similar findings [39, 42] confirm that thermophilic bacteria producing α-amylase and protease isolated from hot springs was belonging to *Bacillus licheniformis*, *Bacillus aerius, Bacillus subtilis* and *Bacillus sonorensis*.

There is an urgent need to decrease food waste in Saudi Arabia and find new and efficient strategies for recycling and utilization [12]. The chemical composition of Kitchen wastes (KW) indicates that the main components of organic matter, fat, protein and carbohydrates were present in high proportions in KW [43]. Due to its nutrient-rich nature, kitchen waste can be converted into valuable bio-products such as ethanol, enzymes and organic acids through fermentation processes [13]. Thus, optimization of the production of enzymes (α-amylase, protease and lipase) by selected promising bacterial strains using KW as a carbon source was investigated. The results indicated that optimization of conditions is critical if the fermentation process is to be successful. Maximum enzyme production was obtained at the start of the stationary phase, 72 h after culture initiation, which suggests that enzyme production and bacterial growth correlate. The results show that maximal protease and lipase production followed the growth of the microbes *Bacillus licheniformis* and *Bacillus sonorensis*, but with a certain delay that is probably due to the time needed for the microorganisms to adapt to the waste [44]. The growth-dependent enzyme production facilitates more effective process control along with more efficient use of substrates during fermentation, primarily because undesirable effects of cell metabolism such as catabolic repression are excluded [45, 46]. The reduction in enzyme production after an optimum incubation period can be explained by cell autolysis, nutrient exhaustion and accumulation of enzyme repressors [47]. The initial pH of the medium and incubation temperature both influence enzymatic production by affecting transport across the cell membrane and control of enzyme gene expression [48]. Our findings were in accordance with those of Khalil [38], who reported that thermophilic bacteria isolated from Al-Khubah and Al-Ardah hot springs were lipase and amylase producers whose degree of enzymatic production varied at pH 7.5 to 8.5 and temperatures between 55 and 60°C. The results also suggested that the production of protease by *Bacillus licheniformis* occurs optimally at pH 8.5 and 60°C. These data were similar to those of Zilda et al. [49], who found that the optimum temperature for protease production by *Bacillus licheniformis* isolated from Indonesian hot springs was 60°C and that the optimum pH was approximately 8.0. The production of α-amylase by *Bacillus aerius* using KW was similar, with protease production occurring at an optimum temperature of 60°C, while the optimum pH was slightly alkaline at 7.5. Similarly, the ability of *Bacillus* sp. isolated from hot springs to degrade native starch at a wide range of pH and the thermal stability of α-amylase are attractive attributes, which make this bacterial strain a potential candidate for starch hydrolysis [50]. Moreover, Rekadwad [33] found that optimum lipase production was achieved at 65°C for thermophilic bacteria isolated from hot springs. They also reported that incubation temperature affects all the physiological activities in a living cell and is an important environmental factor for controlling growth, microbial activities and normal functioning of an enzyme. Hydrolytic enzymes are relatively costly, and a significant reduction in production cost is required if they are to be used commercially.

From an economic point of view, there is a need to increase enzyme production by using cheaper substrates such as kitchen waste [9]. The results showed that high levels of enzyme production were achieved from KW without any further treatments. The α-amylase and protease production was optimal at 7% KW, while for lipase production, a 5% KW concentration was favourable. The presence of lipids in the KW medium induced lipase production; however, the high concentrations resulted in lower secretion as a result of inhibition of microbial growth and reduced oxygen availability [51]. The decrease in α-amylase production at higher substrate concentrations can be explained by the production of reducing sugars, which inhibit enzyme activity [52].

5 Conclusion

Thermophilic bacterial strains were successfully isolated from different hot springs in Saudi Arabia. These isolates can produce industrially important enzymes needed for

the hydrolysis of starch, proteins and lipids with elevated activity from cheap and unexploited environmental wastes. The results suggest that these industrial enzymes can be produced using kitchen waste, resulting in high enzyme productivity without any further treatment.

Conflict of interest: Authors state no conflict of interest

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