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RESEARCH ARTICLE OPEN ACCESS

Determination the Lipase Activity of *Staphylococcus sp.* Strain Isolated from Clinical Specimens

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Abstract

Bacterial lipases are the most important collection of biocatalysts used for a variety of biotechnological applications. In the current study the morphological, biochemical and Molecular characteristics to the extracellular lipase producing bacteria were identified. The bacterial isolates were selected as lipase producing bacteria using Rhodamine-B agar plate. the production of Lipase from the organism were determined with varying incubation temperature (20 to 50°C) and range of incubation time (16-120) hrs. 16 bacterial isolates were identified as *Staphylococcus hemolyticus* strain SHKS1 with Genbank acc. MK977614.1. By comparing the observed DNA sequences of these local samples with the retrieved DNA sequences (GenBank acc. MG595372.1) as showed high lipase activity according to experimental conditions about 140 U/ml. The current study showed that enzymatic activity of crude lipase extract was maximum in 72 hrs incubation period and 37°C.

Keywords: Lipase, *Staphylococcus hemolytic*, Genetic Identification, Lipase extraction, Enzyme activity

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INTRODUCTION

Lipases were occupied a special situation as biotechnologically valuable enzymes and display vital roles in various industries[1]. Lipases occur widely in nature and only microbial lipases are substantial. Microbial lipases are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activities available, the high yields possible, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media[2]. Lipase is an enzyme with considerable commercial and industrial importance. Lipase (triacylglycerol acyl hydrolases (E.C.3.1.1.3) belongs to the class of hydrolases which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil-water interface[3]. Lipases catalyze the reverse reactions in the synthesis of esters (triacylglycerols) consist of glycerol and fatty acids of long-chain in non-aqueous circumstances[4]. The activities of All lipolytic enzymes are depended on the pattern of α/β- hydrolase fold, However, the lipases from microbial origin specially show a distinctive folding design of α/β- hydrolase fold[5,6]. The greater part of industrial enzymes are produced from microbial source, they are more stable and constitute a superior diversity of catalytic actions[7].

Staphylococci spp. release extracellular lipases in a fermentation medium were encompassed remedying and degrading enzymes in the environments[8]. The present study aimed to determine the production of lipase from bacterial isolates by investigation the produced lipase from clinical specimens and determination its activity in these isolates.

MATERIAL AND METHODS

Isolation of lipase producing isolates

The clinical isolates included in the present study were collected within a one-month period. The isolates were obtain from unite of clinical microbiology in the hospital training in the governorate. These isolates were taken from primary isolation plates set up on solid media such as blood agar, MacConkey agar and mannitol salt agar. The bacterial colonies which observed on nutrient agar medium were isolated to identification and selected for screening lipase activity[9].

Identification of bacterial isolates

The morphological, biochemical and genetic features of isolates were identified according to Bergey's manual of determinative bacteriology.

DNA Sequencing of PCR amplicons

The bacterial isolate which shows maximal lipase activity on ROA plates assay is considered as positive colonies for lipase enzyme production, the isolates were identified according to molecular genetic study by using of *16SrRNA* gene sequencing.

The resolved PCR amplicons were sequenced from both termini of forward and reverse based to the sequencing company instruction manuals (Macrogen Inc. Geumchen, Seoul, South Korea). By comparing the observed DNA sequences of local bacterial samples with the retrieved neighboring DNA sequences of the NCBI Blastn engine.

The results of the PCR products sequencing of the isolate was analyzed as long as with the respective sequences in the reference database. The observed variations in each sequenced sample were numbered in PCR amplicons as well as in its corresponding position within the referring genome.

A specific comprehensive bacterial tree was constructed in this study according to the protocol described by[10]. The observed bacterial variants were compared by reference sequences neighbor homologous by means of NCBI-BLASTn server[11]. Then, the results of BLAST of the observed variations were linked and aligned together using a Clustal Omega based tools. A full inclusive tree, including the observed variant, was imagined according to (http://tree.bio.ed.ac.uk/software/figtree/)[12].

Lipase producing bacteria investigation

The Rhodamine B- olive oil-agar medium (ROA) was used to select the lipase producing isolates. The medium was prepared according to[13]. The isolates were identified after 48h of incubation at 37°C and exposed to U.V. light at 350 nm[14]. The isolates were culture overnight in liquid medium was prepared according to[15].

The standard liquid medium for measurement the lipase activity according[16] use for culturing lipase producing bacteria. the broth media were inoculated of 5% overnight culture

Table 1. The position and length of the 1429 bp PCR amplicons used to amplify a portion of 16S rRNA locus of Staphylococcus haemolyticus genomic DNA sequences (GenBank acc no. MG595372.1).

Amplicon	Referring locus sequences (5′ - 3′)	length
16SrRNA sequences	ATACATGCAGTCGAGCGAACAGATAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGAATAA- CTCCGGGAAACCGGGGCTAATGCCGGATAACATATAGAACCGCATGGTTCTATAGTGAAAGATGGTTTTGCTATCACTTATAGATGGACCCGCGCCGTATTAG- CTAGTTGGTAAGGTAATGGCTTACCAAGGCGACGATACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGG- GAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGGTTTCGGCTCGTAAAACTCTGTTATTAGG- GAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA- GCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGG- AAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTG- TAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCG- CCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTG- GAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTTCCCCTTCGGGGGACAAAGTG- ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGC- ACTCTAGGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAA- TACAAAGGGCAGCTAAACCGCGAGGTCATGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGT- AATCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACC- ATTTATGGAGCTAGCCGTCGAA	1429 bp

media of all the lipase producing isolates was prepared according to[17], the medium consist of (g/L): yeast extract, 2.5; pepton, 5.5; NaCl, 10 and 1% olive oil, autoclaving at 121°C for 15 min. The media incubated at 150 rpm, 37°C and pH value 6 [18].

Lipase crude extraction

The crude lipase was extracted according to[16].

Lipase assay

The titrimeteric method was used to measure the activity of lipase based on[18]. using olive oil as substrate.

Effecting the incubation period and incubation temperature in lipase production by isolate Staphylococcus hemolytic

A selection of isolate Staphylococcus hemolytic was cultivated in fermentation medium for five day. varying from 20°C to 50°C of incubation temperature were selected to determine the optimum temperature for lipase production, while other limits keeping at same[19].

RESULTS AND DISCUSSION

The laboratory and genetic identification

The technique of Enrichment culture enable the isolation strains of bacteria from different source. The results in the current study appear that the supposed isolate was *Staphylococcus hemolytic* according to its' identifying as Gram positive, cocci in clusters and tests made for catalase and coagulase production, allowing the coagulase-positive *staphylococci* to be identified quickly[20].

Within this locus, The present study were included the sample that had shown to amplify 1429 bp genetic sequences in *Staphylococcus* haemolyticus genome. The strain " St1" was identified as *Staphylococcus* haemolyticus strain SHKS1 with Genbank acc.MK977614.1. The sequencing reactions indicated that the exact identity after performing NCBI blastn for these PCR amplicons "https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch". Concerning the supposed ribosomal 1429 bp amplicons, NCBI BLASTn engine shown about 100% sequences similarities between the sequenced samples and the intended reference target sequences. By comparing the observed DNA sequences of these local samples with the retrieved DNA sequences (GenBank acc. MG595372.1), the approximate positions and other details of the retrieved PCR fragments were identified (Fig. 1).

After positioning the 1429 bp amplicons' sequences, the details of its sequences were highlighted, in terms of the positioning of both forward and reverse primers of the 1429 bp amplified amplicon (Table 1).

The alignment results of the 1429 bp samples revealed the absence of any detected polymorphism in this position in the investigated sample in comparison with the referring reference DNA sequences (Fig. 2).

A comprehensive phylogenetic tree was constructed, (65) sequences were comprised the total number of the aligned nucleic acid sequences, irrespective of the investigated local bacterial variant. The total number of the included species within this tree was made of six species within *Staphylococcus* genus, namely *S. haemolyticus, S. gallinarium, S. arlettae, S. xylosus, S. succinus,* and *S. saprophyticus*. Though the current constructed comprehensive tree indicated the presence of several species one, one genus was detected allover this comprehensive tree (Figure 3). However, it was revealed that the investigated

Fig. 1. The exact position of the retrieved 1429 bp amplicon that partially covered a portion of 16S rRNA locus of *Staphylococcus haemolyticus* genomic DNA sequences (GenBank acc no. MG595372.1).

Fig. 2. DNA sequences alignment of the local sample of 16S rRNA with their corresponding reference sequences of the 1429 bp amplicons within *Staphylococcus haemolyticus* genomic DNA.

St1 was positioned in a unique position beside KT026096.1 and AB726090.1 accession number which they belonged to *S. haemolyticus* and *S. gallinarium* respectively. This observation gave another confirmed ideation with regard to the accurate identity of *S. hamelotyicus*. However, the unique position occupied by the observed S1 between *S. haemolyticus* and *S. gallinarium* has indicated a particular positioning of the St1 in terms of its phylogenetic association with the other related species within the *Staphylococcus* genus. However, this multi-species positioning did not mean the inability of this 16S- based ribosomal amplicon in the detection of the mainly targeted *S. haemolyticus* species. Moreover, the currently utilized ribosomal primers may not reflect high specificity of the targeted site as five other non-targeted organisms were revealed in the immediate vicinity to the investigated St1 isolate. This is due to the fact that St1 has characterized with unique nucleic acid sequences that made it localize in this phylogenetic position beside several related nucleic acid sequences that are belonging to other organisms of the same genus.

Investigation, screening of lipase producing bacteria

The investigation of lipase activity to the screened isolates were showed in table 2 and Fig. 4, It appears that the colonies with lipolytic activity showed orange florescence halos after growth on Rhodamine -B agar plates test for 48hr of incubation. The results showed that one bacterial isolate St1 from Sixty isolates were characterized by this activity during the present study.

The hospital environment is one of different sources for a microbial population. As shown in Fig. 4 the colonies of *S. haemolyticus* (St1) showed positive on Rhodamine -B agar plates test. The colonies of lipase producing bacteria were showed halos of orange fluorescent during 48hrs of incubation at 37°C, This appearance may be due to the hydrolysis of substrate, or due to the construction of conjugate of a Rhodamine -B long chain fatty acid. The results in the present study agree with study of[21,13,22-26]. Qualitative screening of *Pseudomonas* sp LSK25 lipase showed positive evidence of lipase production. The strain was able to produce orange fluorescent halos (Rhodamine B) within 48 h incubation at 4°C 27.

Determination of lipase activity

The results of effect incubation time in the lipase construction is appear in the Fig. 5. As it shown in this figure the maximum production of crude lipase extract for the isolate *Staphylococcus haemolyticus* in 72hrs with

Table 2. Lipase activity on ROA plate test for isolates Staphylococcus haemolyticus

Symbol of isolate	Result of ROA test
St1	+
St2	–
St3	–
St4	–
St5	–
St6	–
St7	–
St8	–
St9	–
St10	–
St11	–
St12	–
St13	–
St14	–
St15	–
St16	–

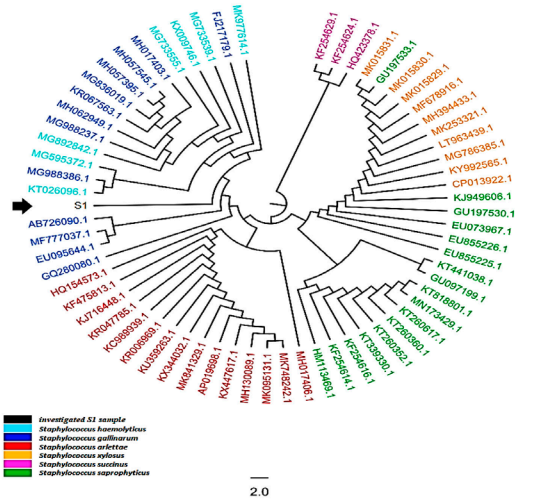

Fig. 3. The comprehensive phylogenetic tree of the 16S rRNA genetic fragments for *Staphylococcus haemolyticus* local isolates. The black color and arrows refer to the sequenced query local samples, while other colors refer to other referring NCBI Staphylococcus deposited referring species.

fixing other experimental conditions was about 140U/ml. The extracellular lipase production of *Staphylococcus* spp. were affected by number of factors. The present study agree with[28], who suggest that 48hrs of incubation was the optimum period for lipase production. The study of[29,30] showed that the maximum lipase production was found at 48hrs. of growth for Bacillus spp. The study of[30] showed that the maximum production of lipase being highest than 24U/ml after 48hrs. of incubation. According to[19], The maximum lipase activity by S. aureus of the culture medium was seen after 45 hours at 35°C. While the study of[27] reported that The highest lipase activity was achieved over 36 h at 10°C in production medium at pH 7.0.

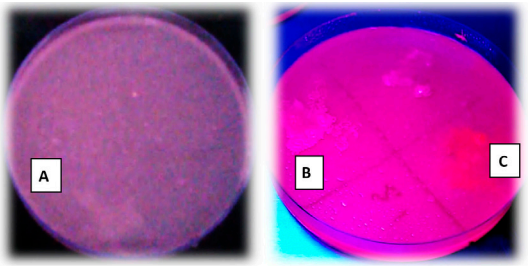

Fig. 4. Lipase activity on ROA plate .. A: Control, B: Negative test, C: "St1" positive test

The results of effect of the incubation temperature is appear in the Fig. 6. It was showed that 37°C the optimum temperature for lipase production. The synthesis of enzyme is control by the incubation temperature of fermentation medium at mRNA transcription level and probably translation levels of enzymes thus increases the stability of enzymes and enzyme production. However the optimal temperature for lipase production agrees with the growth temperature of the microbes[23]. also the present study suggest that the production of lipase at high temperature due to that temperature stimuli the secretion

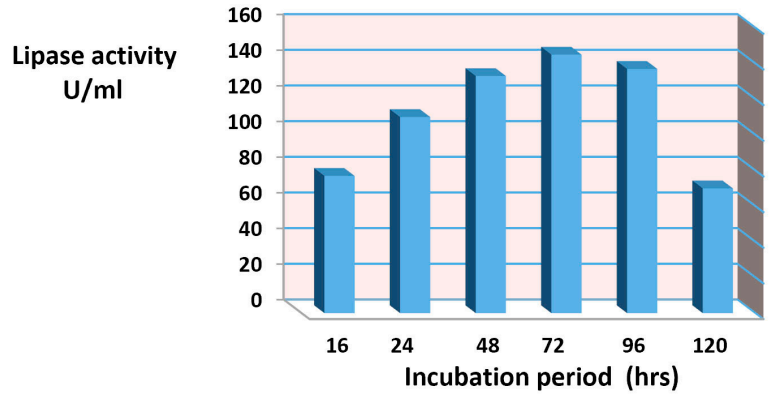

Fig. 5. Effect of Incubation period in lipase production

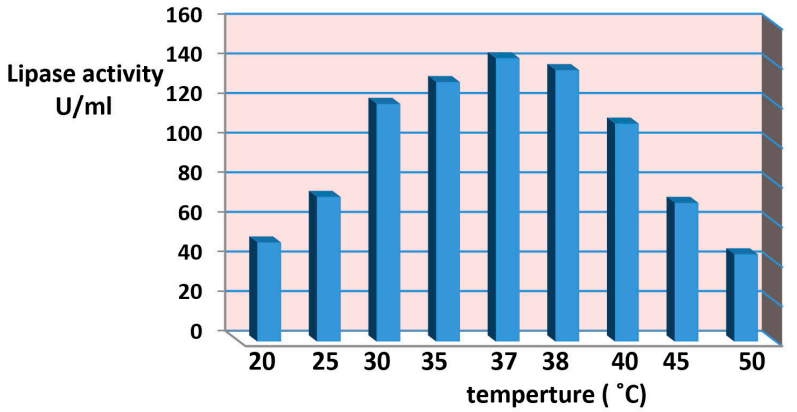

Fig. 6. Effect of temperature ranges in lipase production

of extracellular enzymes by shifting the physical characteristics of the cell membrane[23]. The study of[31] reported that the effect of temperature on enzyme activity is attributed to its effect on stability, rate of the reaction, substrate solubility as well as direct influence on esterification reaction. The results in the present study agree with[9,32] it reported the optimum incubation temperature for lipase production at 37°C of *Pseudomonas gessardii* and certain species of *Pseudomonas*. While the study of[33-35] were reported the incubation temperature at 25°C was gave the maximum lipase production for *Pseudomonas* spp., While the study of[36] whose exhibited that 40°C the maximum lipase production for *Pseudomonas cepaciea* was at 40°C. and the study of[37,38] reported that incubation temperature at 50˚C was optimum temperature for production of lipase by Bacillus sp. 8°C. The study of[39] reported that the showed that the most suitable temperature for the highest lipase activity (225 U/ml) was 10°C, whereas the lowest lipase activity (175 U/ml) was detected at 30°C.

CONCLUSION

Staphylococcus hemolytic is prosperous bacteria has ability to secrete extracellular lipase. The bacterial isolates from clinical sources were screened as lipase producing bacteria and showing on Rhodamine-B agar plate. Lipase production of organism were measured with varying incubation temperature (20 to 50°C) and incubation time range (16-120)hrs. The present study displayed that *Staphylococcus hemolytic* (GenBank acc. MG595372.1) isolated of the clinical specimens described in high lipase production. The results appeared that the enzymatic activity of crude lipase extract was maximum for incubation period of 72 hours at 37°C about 140 U/ml.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

AUTHORS' CONTRIBUTION

HJJA contributed by measurement of lipase activity and the genetic analysis for the isolates. AKA contributed by isolation and identification of the isolates. BMM analyzed the data and helped for preparation of figures, interpretation and draft.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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