Identification of Lipase Producers*Staphylococcus aureus* and Studyingthe Effects of Some Physicochemical Factors on Lipase Production

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ABSTRACT

Lipases catalyze both the hydrolysis and synthesis of triacylglycerols. In addition to the industrial uses of lipases, there is an evolving literature on their role as important microbial virulence factors. One hundred samples were collected from different body sites and lesions of in and out patients from both sexes who attended Al-Kadhimyia Teaching Hospital and Medical Laboratories in Baghdad Teaching Hospital during the period from March, 2012 until September, 2012 for the isolation and identification of *Staphylococcus aureus*. Identification of bacterial isolates was performed according to the standard protocols. According to lipase activity measurement, it was revealed that isolate no. 12 was the higher producer as compared to the others and thus it was selected for further work.The results demonstrated that the best lipase production was in the alkaline pH (8) and at temperature of 30^{o}C. It is also shown that the addition of 0.001M $BaCl_2$ and 2mM KCl to the culture media has dramatically increased lipase activity. Conversely, the addition of 10mM NaCl, 0.001M $FeSO_4.7H_2O$, and 2M NaF has caused significant reduction in lipase activity.

تشخيص عزلات المكورات الذهبيه العنقوديه المنتجه لانزيم اللايبيز ودراسة تاثير بعض العوامل الفيزيوكيمياويه على انتاج اللايبيز

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الكلمات المفتاحيه: بكتريا المكورات الذهبيه العنقوديه, انزيم اللايبيز, الايونات اللاعضويه, الفعاليه الانزيميه

الخلاصة

يحفز انزيم اللايبيز عملية التحلل المائي والتخليق لمركبات ثلاثية الاسل الكليسورليه وبالاضافة الى الاستعمالات الصناعية للايبيزات فان هنالك العديد من المصادر التي تشير الى اهميتها كعوامل ضراوه مهمه للمكروبات. تم جمع مئه عينه من اماكن وآفات مختلفه من جسم الانسان للمرضى من كلا الجنسين الذين زاروا العياده الخارجيه او كانوا راقدين في مستشفى الكاظميه وبغداد التعليميين للفتره من اذار لسنة 2012 ولغاية ايلول من نفس السنه. وكان الغرض من جمع هذه العينات هو لعزل وتشخيص بكتريا المكورات العنقوديه الذهبيه.تم تشخيص العزلات الاولي بالطرق الكيموحيويه وبالاعتماد على الطرق القياسيه وبقياس فعالية انزيم اللايبيز تبين ان العزله رقم 12 هي الاعلى في انتاجية هذا الانزيم مقارنة بالعزلات الاخرى ولذلك تم اختيارها لاجراء باقي التجارب. .اظهرت نتائج الدراسه ان افضل انتاج لانزيم اللايبيز كان في البيئه القاعديه (pH 8) وعند درجة حراره 30 مئويه كما تبين ان اضافة 0.001 ملي مولر من ثنائي كلوريد الباريوم و 2 ملي مولر من كلوريد البوتاسيوم تؤديان الى زياده ملحوظه في فعالية انزيم اللايبيز على العكس من اضافة 10 ملي مولر من كلوريد الصوديوم و 0.001 ملي مولر من حامض الكبريتيك المائي و 2 ملي مولر من فلوريد الصوديوم التي ادت الى انخفاض ملحوظ في فعالية انزيم اللايبيز.

1. INTRODUCTION

Staphylococcus aureus is a ubiquitous bacterium that is generating increasingly bad press coverage due to its propensity to adopt a pathogenic lifestyle in hospital and community settings.[1]Lipases catalyze both the hydrolysis and synthesis of triacylglycerols. In addition to the industrial uses of lipases, there is an evolving literature on their role as important microbial virulence factors. [2] *S. aureus* colonies are found in approximately 30% of the general population [3] and causes pyogenic infection in man and is the common cause of boils, carbuncles, impetigo and infection of surgical or accidental wounds and burns.[4] Various forms of trauma resulting around superficial abscess such as squeezing a boil may force large number of staphylococci into the blood from where they are carried into many tissues of the body; thus, systemic infections and abscesses may be produced in various deep organs such as liver, lung or brain tissues.[5]

The putative roles of microbial extracellular lipases include digestion of lipids for nutrient acquisition, adhesion to host cells and host tissues, synergistic interactions with other enzymes, nonspecific hydrolysis due to additional phospholipolyticactivities, initiation of inflammatory processes by affecting immune cells, and self-defense mediated by lysing competing microflora.[6]

The aims of the study is to identify lipase producer *S. aureus* and study the effects of pH, temperature, and addition of inorganic ions on lipase production; for these parameters to further manipulate the produced lipase for eventual practical applications.

2. MATERIALS AND METHODS

One hundred samples were collected from different body sites and lesions of in and out patients from both sexes who attended Al-Kadhimyia Teaching Hospital and Medical Laboratories in Baghdad Teaching Hospital during the period from March, 2012 until September, 2012 for the isolation and identification of *S. aureus* which was identified through microscopic detection, cultural examination, and biochemical tests.API Staph system was applied for confirming the primary bacterial identification.The method described by Bier [7] for the measurement of lipase activity through the titration of resulted fatty acids with 0.05 N sodium hydroxide was applied.Solution of reaction was prepared according to Aisakaand Terada.[8]Enzyme activity was calculated according to Varley and Bell [9].Isolate No. 12 showed the higher lipase activity among all other isolate thus it was selected as a candidate for further work.

Ten screw capped universals were prepared with 10 ml of brain heart infusion broth inside each, then the pH of the media in each universal was adjusted to4.5,5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9. Each screw capped tube was inoculated with *S. aureus* isolate no. 12 and incubated for 24 hrs.Afterwards tubes were collected and enzyme activity was measured for each tube separately.To determine the optimum temperature for lipase production, six screw capped tube each loaded with ten milliliters of brain heart infusion broth were inoculated with lipase producer *S. aureus* isolate no. 12and were incubated with different degrees of temperature (25, 30, 35, 40, 45, and 50^{o}C) for 24 hrs. [10]

Another six screw capped tubes were prepared to have ten ml brain heart infusion broth each; tubes one through five contained 10mMNaCl, 0.001M $BaCl_2$, 2mMKCl, 0.001M $FeSo_4.7H_2O$, and 2M Naf, respectively. Tube number six contained only the culture media and

left untreated as control.The tubes were inoculated with lipase producer *S. aureus* isolate no. 12 and were incubated for 24 hrs. Enzyme activity was measured for each tube separately. [10]

3. RESULTS

From a total of 100 specimens enrolled in the current study, *S. aureus* constituted 50 % of all specimens and it was isolated as a common pathogen that can cause serious infections in various body sites and tissues (Table 1).

Table 1:Ranking of *S. aureus* and the percentage of each infection from a total of 50 cases.

Cases	No. of isolates	%
Wounds swabs	15	30%
Ear swabs	5	10%
Sputum	10	20%
Urine samples	6	12%
Urethral discharge	4	8%
Vaginal swabs	3	6%
Blood samples	7	14%
Total	50	100%

Lipase activity was assayed for all the fifty isolates enrolled in the current study and it appeared that isolate number 12 showed the maximum enzyme activity (600 □mole/ml) as compared to all other isolates (Figure 1).

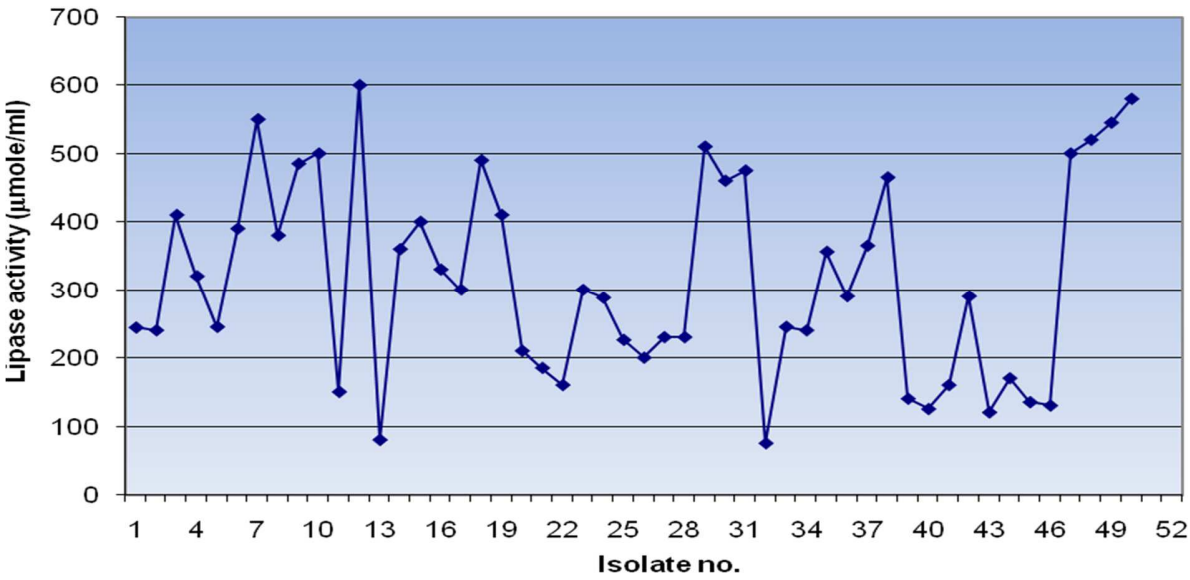

Figure 1: Isolates of *S. aureus* with different lipase activities.

The results indicated that lipase production was maximal at pH near alkalinity (8) as indicated by an enzyme activity of 540 □mol/ml and it was also clear that lipase production was significantly reduced as the pH of the medium shifts gradually towards acidity but showed little effect when pH progressed towards alkalinity (Figure2).

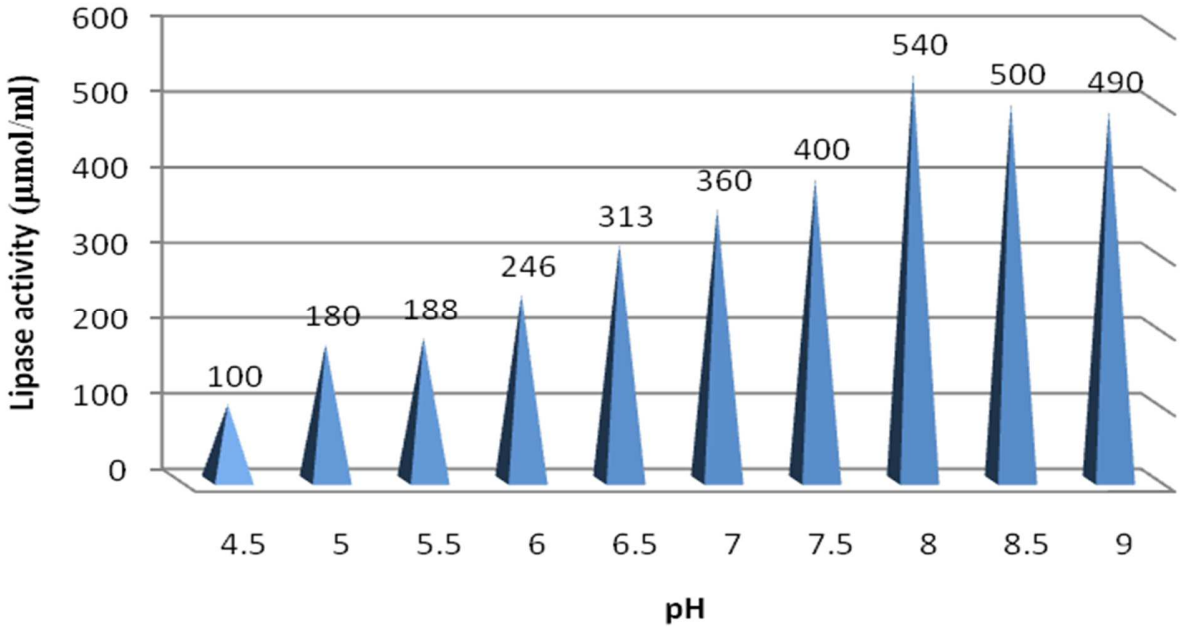

Figure2: Effect of the pH on production of the lipase from the local isolate of *S. aureus* (12).

A range of incubation temperatures was applied to specify the optimum temperature of incubation. The results showed that the maximum enzyme activity (1115 mole/ml) was obtained when incubation temperature was 30°Cfor a period of 24 hrs (Figure 3).

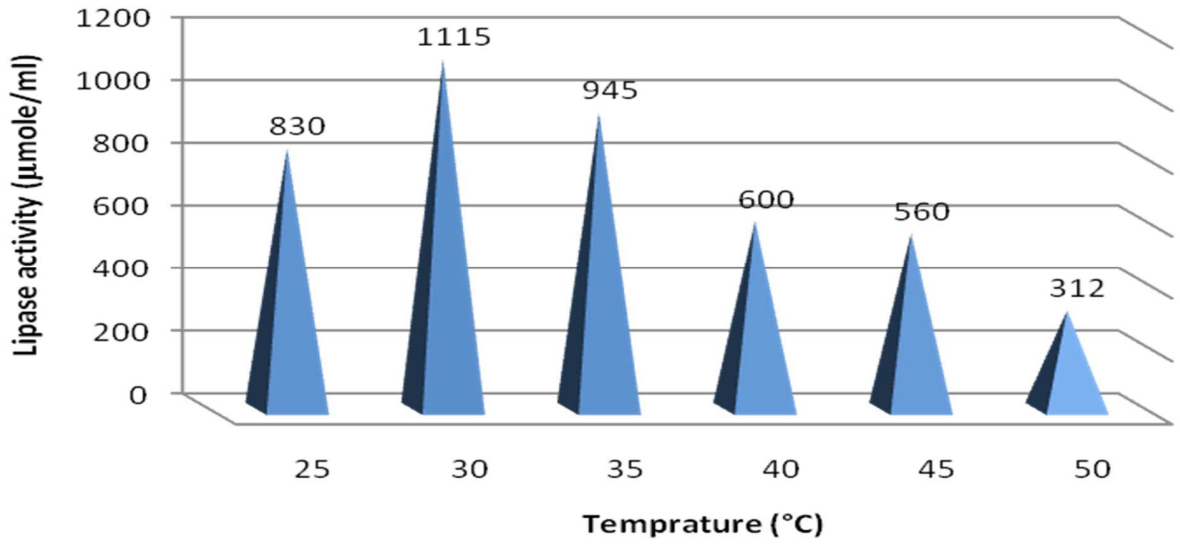

Figure3: Effect of temperature on the production of lipase from a local isolate of *S. aureus* (12).

Five inorganic ions were selected to evaluate their effects on lipase activity. It was shown that the addition of $BaCl_2$ and KCl had dramatically increased lipase activity, while the addition of NaCl, $H_2SO_4.7H_2O$, and Naf had negative influence on lipase activity as compared to the control (Figure 4).

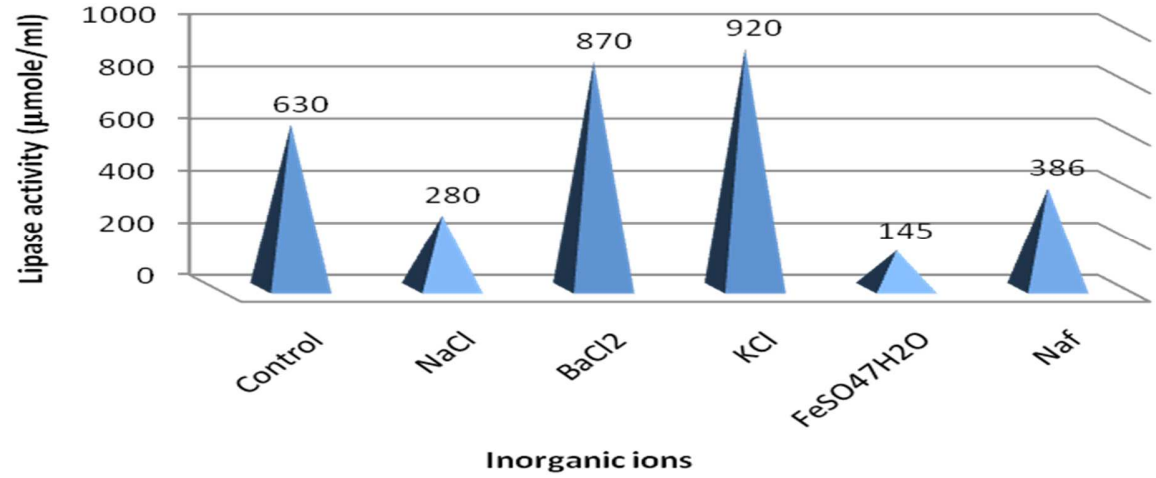

Figure 4: Effect of inorganic ions on the production of lipase from a local isolate of *S. aureus* (12).

4. DISCUSSION

Staphylococci remain among the most important pathogens isolated in the clinical microbiology laboratory [11]. *S. aureus* was superabundant and easy to collect from different body sites and lesions because of the following reasons: it's a leading cause of hospital-acquired infections and it is the primary cause of lower respiratory tract infections, pneumonia surgical site and cardiovascular infections[12,13] and the second leading cause of nosocomial bacteremia [14]. Infections with *S. aureus* are especially difficult to treat because of evolved resistance to antimicrobial drugs [15].

Data presented in Table 1 showed that *S. aureus* consisted of 50% of all enrolled isolates. Similar results were obtained by other workerswho reported percentages that ranged between 27.6% to 67.3% indicating that this bacterium was a dominant pathogen.[16, 17]The differences in the frequency of isolation could be attributed to factors such as the anatomical site of infection, type of lesion, age, and geographical area of isolation.[18]

Our results showed that the maximum enzyme activity was 600 mole/ ml. The ability of microorganisms to produce enzyme is influenced by environmental conditions such as temperature, pH and presence of inducers or repressors.[19] The results also indicated that lipase production was maximal when the pH of the medium was alkaline (pH 8).In agreement with the results of the current study, an alkaline pH 8.5 was optimal for maximum lipase activity produced by *P. fluorescens* in medium which contained emulsified olive oil as the carbon source,[20] while the study of Sztajer*et al*., [21] contradicted the results of the present study when it was found that maximal production of extracellular lipase from*P. Citiinum* was at pH 7.2. Interestingly, researchers working with lipase from *Aspergillusoryzae* recorded higher enzyme activity when the medium approached prominent acidity of pH 4.0 [22].

Bacterial lipases having neutral [23], basic[24] and acidic[25] optimum pH have been reported by different workers. It was found that bacterial lipases show different degrees of enzyme activity in relation to the type of enzyme produced by the microorganism and it was also stated that the optimum pH for bacterial growth and enzyme activity is not necessary match. The pH of the medium can affect the ionizing groups found in the enzyme substrate complex and this will lead to decreased saturation of the enzyme active site with the substrate or a decrease in enzyme stability or both resulting in altered enzyme stability under changing degrees of pH [26].

The results of the current study revealed that the activity of the enzyme peaked at temperature 30^{o} C, and it also revealed that the higher temperature the greater the drop in the activity of the enzyme, while the drop in the activity of the enzyme was less when the condition of incubation directed towards colder degrees. This might be explained due to limited adaptation of the enzyme to a reasonable increase in temperature. Many studies were held to detect the optimum temperature for lipase production and some of these results were similar to the results of the present study. Our results are similar to that obtained by Dong *et al*., [27]who were working with *pseudomonas* and stating that optimum lipase activity attained at 30°C for 72 hrs of incubation.In a previous study by Sidhu *et al*.,[28] an extra cellar *Bacillus* lipase had optimum activity at 50 °C, while Wang *et al*., [29] showed that the maximum enzyme activityfrom *Bacillus* strain was achieved at 60°C.The results of the present study contradict that of Tyski *et al*., [30] who found an optimum temperature for lipase activity from staphylococci to be around 55^{o}C.

The marked decrease in enzyme activity at decreased temperature of incubation can be explained by the fact that under low temperatures bacterial growth is suboptimal and the biosynthesis of enzymes is at its lowest level, while normal enzyme production is restored at temperatures close to the forties due to the ability of bacteria to grow well around these degrees.[31]

In respect to the selected inorganic ions, it was shown that addition of KCl and $BaCl_2$ had increased lipase activity, while addition of NaCl,H_2SO_4.7H_2O and Naf had negative influence on lipase activity. Chartrain *et al.*,[32] observed that an extracellular lipase of *P.aeruginosa* was stimulated by $CaCl_2$, while the activity was not affected by Mg^{+2}, Mn^{+2}, Na^{+} and K^{+}. It was proposed that calcium is needed as a cofactor for catalytic activity.[33]

The inhibitory effect of certain ions on lipase activity of *P. fragi* was observed by Nashif and Nelson [34]. Also, it had been noticed that some ions (Zn^{++} and Hg^{++}) hadan inhibitory effect on lipase activity.[35]It was observed that $SrCL_2$, $BaCl_2$, and $MgCl_2$ were the best activators of lipase and this finding was explained by the absolute requirement shown by most lipases to fatty acids acceptors (divalent cations) [36].In a marked agreement with the results of the present study, a researcher also showed that lipase activity was enhanced by the addition of KCl to the culture medium of staphylococci. The stimulatory effects of salts can be explained by the fact that they can facilitate or interfere in the interaction between active binding site of the enzyme and the substrate, while the inhibitory effects of salts on enzyme activity may be due to the changes in the ionic strength of the environment which can affect the ionic state of both the enzyme and substrate.[7]In another study that agreed with the results of the present one, three forms have been purified and characterized and found to have their activity strongly inhibited by Ag^{+2} and Hg^{+2}, whereas Ca^{+2} and Mg^{+2} enhance lipase activity.[37]In Algerian study conducted at Sidi bel abbes on samples collected from waste water it was mentioned that the activity of lipase produced by *P. aeruginosa* was enhanced by Ca^{+2} and Mg^{+2} but strongly inhibited by heavy metals Zn^{+2}, Cu^{+2} and Mn^{+2}[38]which is in agreement with the results of the present study.

CONCLUSIONS

S. aureus was identified as a major pathogen affecting multiple body organs and tissues and can cause considerable health problems and that lipase production was prominent among isolates belong to this species. It is concluded that lipase production was affected by changing some physicochemical parameters.

REFERENCES

[1] RussellDG. (2008) . *Staphylococcus* and the Healing Power of Pus. ***Cell Host & Microbe.*****3**(3): 115-6.

[2] Bramono K, Yamazaki M,Tsuboi R, and Ogawa H. (2006) Comparison of proteinase, lipase and alpha-glucosidase activities from the clinical isolates of *Candida* species.***Japan J. Infect. Dis.*** (59):73–76.

[3] Doebbeling BN. (1994) Nasal and hand carriage of *Staphylococcus aureus* in healthcare workers. ***J. Chemother.***6:11.

[4] Jacquelyn GB. (2000)*Staphylococcus aureus*. In: Text book for Microbiology, 4th Ed. Pp: 523-524. John Wiley & Sons. USA.

[5] HidaK,Yanai S, Shimizu K,*et al*., (2005) A case of liver abscess with secondary brain and lung abscess in a healthy man. ***J. Japan Surg. Assoc.*** 66: 1990-1993.
[6] Schaller M, Borelli C, Korting HC, and Hube B. (2005) Hydrolytic enzymes as virulence factors of *Candida albicans*. ***Mycoses.*** (48):365–377.
[7] Bier M. (1955) Lipases In: ***Methods in Enzymology.*** (1): 103-107.
[8] Aisaka K and Terada O (1979) Production of Lipoprotein lipase and Lipase by *Rhizopusjaponicu* .43 (10): 2125-2129.
[9] VarleyG and Bell M (1976) General topics and Commoner tests. In: ***Practical-clinical Bioch.*** 1 (5^{th} Ed.).
[10] Aziz F(2004) "Lipases" The Multifunctional Enzymes.Ph.D.Thesis submitted to the Department of Microbiology/ University of Karachi. Pakistan. p.25.
[11] Richards MJ, Edwards JR, Culver DH, and Gaynes RP. (1999a)Nosocomial infections in medical intensive care units in the United States. ***Crit. Care Med.*** 27: 887–92.
[12] Richards MJ,Edwards JR,Culver DH, and Gaynes RP. (1999b)Nosocomial infections in pediatric intensive care units in the United States.***Pediatrics.***103: 39.
[13] Wisplinghoff H, Bischoff T, Tallent SM,Seifert H, Wenzel RP, and Edmond MB. (2004) Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. ***Clin. Infect. Dis.***39: 309–17.
[14] Lowy FD. (2003)*Staphylococcus aureus* infections. ***N. Engl. J. Med.*** 339: 520-532.
[15]Dzwonkowska J,KurlendaJ,BaczkowskiB,*et al*., (2007). The effect of antibiotic therapy on the incidence of *Staphylococcus aureus* infections inorthopaedic patients. ***Orthop.Traumatol.Rehabil.*** 9(5): 532-47.
[16] Chen CJ, Su LH, Lin TY, and Huang YC. (2010)Molecular analysis of repeated methicillin-resistant *Staphylococcus aureus* infections in children.***Plos.One.*** 5 (12): 10-1371.
[17] Akortha EE, Aluyi HAS, and Enerijiofi KE. (2011) Transfer of amoxicillin resistance gene among bacterial isolates from sputum of pneumonia patients attending the University of Benin Teaching Hospital, Benin City, Nigeria. ***J. Med. and Med. Sci.***: 2(7): 1003–1009.
[18] Gupta RN and RathiP.(2004) Bacterial lipases: an overview of production,purification and biochemicalproperties.***Appl.Microbial.Biotech.*** 64 (6):763-781.
[19] Lee SY, Rhee JS.(1993) Production and partial purification of a lipase from *Pseudomonas putida* 3SK. ***Microb.Technol.***15:617–24.
[20] Ugiura MT, Oikawa K, Hirano and Inukai T. (1977)Purification, Crystalization and Properties of Triacylglycerol Lipase from *Pseudomonas fluorescens*. ***Biochemical Acta.***488:353-358.
[21] Sztajer H,Maliszewska I,and WieczorekJ.(1988) Production of exogenous lipase by bacteria, fungi and actinomycetes.***Enzyme Microb. Technol.*** 10:492–7.
[22] JingZ.(2012)Purification and characterization of lipase produced by *Aspergillusoryzae*.***American Journal of Food Technology***: 596-608.
[23] EmanuilovaEM,KambourovaM,DekovskaandManolov R. (1993) Thermo-alkalophilic lipase producing bacillus selected by continuous cultivation,***FEMS Microbiology letters***.(108):247-250.
[24] KordelMB,HofmannD,Schjcmturgand SchmidRD. (1991)Extra-cellular Lipase of Pseudomonas. Purification, Characterization, Crystallization and Preliminary X-Ray Diffraction Data.***J.Bacteriol***.173(15):4836-41.
[25] Volesky B, and Luong I. (1985) Microbial enzymes production, purification and isolation .CRC (***Critical reviews in Biotechnology***) 2: 119-146.

[26] Oterholm A, Ordal ZJ, and Witter LD. (1970)Purification and properties of a glycerol ester hydrolase (Lipase) from *Propionibacteriumshermanii*.***Applied Microbiology***. 20 (1): 16-20.

[27] Dong H, Gao S, Han S, and Cao S. (1999) Purification and characterization of a Pseudomonas spp. lipase and its properties in non-aqueous media. ***Appl. Microbiol. Biotechnol.*** (30):251–256.

[28] Sidhu P, Sharma R,SoniSK, and Gupta JK.(1998)Production of extracellular alkaline lipase by a new thermophilic *Bacillus* spp. ***Microbiol.***43:51–54.

[29] Wang Y,SrivastavaKC,ShenGJ,and Wang HY.(1995) Thermostable alkaline lipase from a newly isolated thermophilic*Bacillus* strain, A30-1 (ATCC 53841). ***J. Ferment.Bioeng.*** 79:433–438.

[30] TyskiS,Hryniewicz W, and Jeljaszewicz J. (1983) Purification and some properties of the staphylococcal extracellular lipase. ***Biochemical et Biophysicsa Acta***;312-317.

[31] Girmont PAD, and Grimont F. (1984)Genus *Serratia*.In:Bergey`s manual of systematic bacteriology. (Eds. Baltimor, N.R.K.) .Vol.I.pp: 477. Williams and Wilkins.

[32] Chartrain M, Katz L,Marcin*et al*., (1993)Purification and characterization of a novel bio-converting lipase from *Pseudomonas aeruginosa*enzyme. ***Microb.Biotechnology Advances*** (19):627-662.

[33] SimonsJW,VanKampen MD, Riel S,*et al*.,(1998) Cloning, purification and characterization of the lipase from *Staphylococcus epidermidis*—comparison of the substrate selectivity with those of other microbial lipases. ***Eur. J.Biochem.***253:675–83.

[34] Voet D. (1995) Lipid metabolism, In Biochemistry, 2nd Ed. 662-668. John Wiley and Sons, Inc.

[35] Henderson C. (1971)A study of lipase produced by*Anaervibriolipolytica*, a rumen bacterium. ***J. Gen. Microbiol.*** 65:81-89.

[36] GilbertE, Drozd JW, and JonesCW.(1991)Physiological regulation and optimization of lipase activity in *Pseudomonas aeruginosa*. ***J.Gen. Microbiol.***137:2215-21.

[37] Benjamin S and Pandey A. (2001) Isolation and characterization of three distinct forms of lipases from *Candida rugosa*produced in solid state fermentation. ***Brazilian Archives of Biology and Technology.*** 44 (2): 213-221.

[38] Zouaoui B, Bouziane A, and Ghalem B. (2012) Production, optimization and purification of lipase from *Pseudomonas aeruginosa*.***African Journal of Microbiology Research.*** 6 (20): 4417-4423.