Physicochemical Factors Affected the Partial Purified Lipase Activity of *Acinetobacter baumannii* “local isolates”

Huda J. Mohammed[*,1]

[*]Department of Clinical Laboratories Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Microbial lipases today occupy a place of prominence among biocatalysts owing to their ability to catalyze awide variety of reactions in aqueous and non- aqueous media, *A.baumannii* were isolated from different clinical specimens from hospitalized patients from Baghdad hospitals and were detected by biochemical tests and API20E system. The percentage of isolation was (16.6%), *A. baumannii* is an increasingly multidrug – resistant (MDR), it showed high level of resistant to Ceftriaxon, Colistin, Piperacillin, Co-trimoxazol, Tertracycline, Carbenicillin, Amoxicillin, Penicillin G, Gentamicin and Ceftazidim , wherease the isolates were highly sensitive to Imipenem, Ciprofloxacin, Meropenem, Amikacin, and Cefotaxime.

The isolated strians of *A.baumannii* were screened for the production of lipase by using Rhodamine B agar media. one of the isolated strians exhibited a greater clear zone than the others, indicated higher lipase activity.

The lipase in present study was partially purified by ammonium sulphate at the concenterations of 30% and 80%, the 30% ammonium sulphate showed higher activiy of the enzyme than 80% in which the enzyme activity was slightly decreased indicating that the better purification was at the 30% concenteration of ammonium sulphate.Various physicochemical parameters such as pH, temperature and metal ions were studied in order to determine the optimum conditions for lipase production.

The production of lipase by *A. baumannii* was optimum at 35°C, pH 7.0 and was enhanced by the Ca^{++} and Na^{+} wherease inhibited by Zn^{++} ions.

Key words : *Acinetobacter baumannii*, Lipase enzyme, Partial purification.

العوامل الفزيوكيميائية المؤثرة على فعالية انزيم اللايبيز المنقى جزئيا من جرثومة *Acinetobacter baumannii*

هدى جاسم محمد [*،1]

الخلاصة

انزيمات اللايبيز المايكروبية تحتل اليوم مكانة مهمة لدورها الكبير كمحل لات حياتية في الاوساط السائلة وغير السائلة , تم عزل البكتريا من نماذج سريرية مختلفة من مرضى راقدين في مستشفيات مختلفة في بغداد وتم تشخيصها بالاعتماد على الاختبارات الكيموحياتية ونظام(API20E) وكانت نسبة العزل 16,6 %, أن هذه البكتريا واسعة المقاومة للمضادات الحياتية وكانت منها -:
(Ceftriaxon, Colistin, Piperacillin, Co-trimoxazol, Tertracycline, Carbenicillin, Amoxicillin, Penicillin G, Gentamicin and Ceftazidim) .
في حين كانت حساسة للمضادات (Imipenem, Ciprofloxacin, Meropenem, Amikacin, and Cefotaxime).

تم اختبار قابلية البكتريا المعزولة على انتاج أنزيم اللايبيز باستخدام وسط (Rhodamine B agar) وكانت عزلة واحدة فقط منتجة للأنزيم بشكل فعال, تمت التنقية الجزئية لأنزيم اللايبيز بأستخدا م طريقة الترسيب بكبريتات الأمونيوم بتركيز 30% و80%,وأظهر التركيز 30% من كبريتات الأمونيوم فعالية أنزيمية عالية اعلى من تلك عند التركيز 80% وهذا يشير الى ان عملية التنقية الجزئية تكون افضل عند التركيز 30% من كبريتات الأمونيوم.

تم دراسة الظروف الفزيائية والكيميائية التي تؤثر على فعالية الأنزيم وتبين ان الرقم الهدروجيني الأمثل لأنزيم اللايبيز هو 7 ودرجة الحرارة المثلى هي 35 سيليزية وتبين ايضا ان فعالية الأنزيم تتحفز بوجود ايونات الكالسيوم وايونات الصوديوم وتتثبط الفعالية بوجود ايونات الخارصين. .

الكلمات المفتاحية: *Acinetobacter baumannii*، انزيم اللايبيز، التنقية الجزئية.

Introduction

Lipases are the special kind of esterase belonging to subclass 1 of hydrolytic enzyme class 3 and have been assigned sub-sub class 3.1.1 due to their specificity for carboxylic acid ester bonds, these enzymes are widely distributed in nature and have been found in many

Corresponding author E-mail:h.mastermaster@yahoo.com

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species of animals, plants, bacteria, yeast and fungi . Apart from their wide distribution , their presence in microorganisms is most interesting because of their potential application in various industries ranging from the use in laundry detergent to stereo-specific biocatalysts Table 1 [1].

Table 1: List of industrial applications of lipases

Industry	Action	Product of application
Dairy food	Hydrolysis of milk, fat, cheese ripening,modification of butter fat	Development of flavoring agent in milk cheese and butter
Bakery food	Flavor improvement	Shelf life propagation
Beverages	Improved aroma	Alcoholic beverages e.g, sake wine
Food dressings	Quality improvement	Maysoin dressing and whippings
Health food	Transesterification	Health food
Meat and fish	Flavor development	Meat and fish product fat removal
Fats and oils	Transesterification and hydrolysis	Cocoa butter, margarine fatty acids, glycerol mono and diglycerides
Laundry	Reducing biodegradable strains	Cleaning cloths
Cosmetics	Esterification	Skin and sun-tan creams, bath oil etc
Industry	Action	Product of application
Agrochemicals	Esterification	Herbicides such as phenoxypropionate
Pharmaceuticals	Hydrolysis of expoyester alcohols	Produce various intermediates used in manufacture of medicine.
Fuel industries	Transesterification	Biodiesel production
Pollution control	Hydrolysis and transesterification of oils and grease	To remove hard stains, and hydrolyze oil and greases.

The extracellular bacterial lipases are of considerable commercial importance, as their bulk production is much easy [2,3].

Bacterial lipases are greatly influenced by nutritional and physicochemical factors, such as temperature, pH, nitrogen and carbon sources, presence of lipids, inorganic salts, agitation and dissolved oxygen concentration [3].

Each application requires unique properties with respect to specificity, stability, temperature and pH dependence or ability to catalyze [4]. Some stains of *A. baumannii* have the ability to produce different extracellular enzymes including lipases enzymes [5], which are amongst the most widely used biocatalysts due to their ability to catalyze diverse reactions, they reduce fats to fatty acids and glycerol[6].

The genus *Acinetobacter* is classified under the family *Moraxellaceae* and comprises strictly aerobic, gram-negative bacilli, sometimes difficult to decolorize, thus appearing gram positive. It often resemble gram negative cocci in direct smears from positive blood cultures performed in liquid media , while the coccobacillary forms predominate on solid culture media and bacillary forms predominate in liquid media, it is non-motile, lactose non-fermenting, oxidase negative, catalase positive[7].

Acinetobacter spp. are widely distributed in soil, water, sewage, food and can occasionally be cultured from skin, mucous membranes, secretions and from hospital environment, they are part of indigenous microflora of oral and upper respiratory ,genitourinary and lower gastrointestinal tracts as well as of skin [8,9].

Acinetobacter spp. are opportunistic pathogens that readily colonize in patients with compromised host defenses, thereby penetrating deep into the host tissue which serves as a prerequisite for an infection [10]. *Acinetobacter* spp. can cause various types of infections, including pneumonia, septicemia, peritonitis, endocarditis, meningitis, arthritis, corneal infections, urinary tract infections, skin and wound infections and bacteremia[11]. These infections are mainly caused by *Acinetobacter baumannii* , and the mortality rate in patients with *A. baumannii* in intensive care unit fluctuates widely, depending on patient characteristics, such as age and immune status [12]. The risk factors for *A. baumannii* infections include hospital size > 500 beds, include : previous antimicrobial therapy, long stay in an intensive care unit (ICU), catheterization, mechanical ventilation and surgery [13].

Acinetobacter strains are often resistant to antimicrobial agents , and therapy of infection can be difficult, susceptibility test should be done to help select the best antimicrobial drugs for therapy, resistance is more common in *A. baumannii* than in strains of other *Acinetobacter. spp.* [9].

During the last three decades, clinicians are faced with increasing infections of resistant *A. baumannii* strains to almost all clinically applicable antibiotics [14,15], By the late 1990s, carbapenems and polymyxins were only remaining useful agents that could combat severe infections with these organisms in certain hospitals. To date, however, carbapeneme-resistant strains are accumulating worldwide[16], a worrying development gives the paucity of alternative treatment agent[17]. *A. baumannii* respond most commonly to gentamicin, amikacin and to newer penicillins or cephalosporins[9,18].

The specific aim of the current study is: Estimating the optimal pH, temperature, and some metal ions for the better activity of the partial purified lipase from *A.baumannii* to find out its usage in appropriate industry.

Materials and Methods

Isolation and Identification

Specimens of Blood (n=20), Urine (n=20), and Pus from skin lesions (n=20) where collected from hospitalized patients from various hospitals in Baghdad city from the period of March 2012 to July 2012. all specimens were characterized by standard biochemical tests and by API 20 E system.

Antimicrobial susceptibility testing

In vitro activity of the routinely used antibiotics against all isolated strains of *A. baumannii* (n=10) was tested on Mueller Hinton Agar by disk diffusion (Kirby – Bauer Method) [19].

The antibiotics included Imipenem (10 mcg), Ciprofloxacin (5 mcg), Meropenem (10 mcg), Penicillin G (10 mcg), Ceftriaxone (30 mcg), Colistin (10 mcg), Cephotaxime (30 mcg), Piperacillin (100 mcg), Gentamycin (10 mcg), Tetracyclin (30 mcg), Amikacin (30 mcg), Amoxicillin (25mcg), Ceftazidim (30 mcg), Co-trimoxazol (25 mcg), and Carbencillin (100 mcg).

The selection of antibiotics was in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.

Lipase production from A. baumannii

The bacterial strains of *A. baumannii* were grown on nutrient agar containing olive oil (1% w/v) and Rhodamine B 0.001% to assay the production of lipase on Rhodamine B agar plates [20].

Partial purification of lipase enzyme

A. baumannii was grown in nutrient broth for 20 hrs at 37°c.the cell free supernatant, prepared by centrifugation (8000 rpm, 20 min), was passed through a 0.45µm pore size membrane and then ammonium sulphate was added to achieve 30% saturation. The suspension was further centrifuge (8000 rpm, 20 min, 4°c) and the ammonium sulphate was added to the supernatant to reach 80% saturation. The precipitates (0-30%) and (0-80%), collected by centrifugation, were separately dissolved in minimal volume of 20 mM Tris buffer(pH7.0) at 4°c and was dialyzed against the same buffer to remove residual ammonium sulphate[2].

Characterization of lipase lipase assay

The hydrolytic activity was tested by trimetric method, 20 mM phosphate buffer / Arabic gum (1%w/v) and the substrate (olive oil) 1/1 (v/v) were made up to a total volume of 10 ml. The reaction cocktail was thoroughly mixed and equilibrated at 20°c and 1 ml of crude enzyme was added after being previously incubated at the same temperature. The reaction was left at room temperature for exactly 30 min and stopped by adding 3 ml of 95% ethanol. The released fatty acids from oil substrate during enzymatic hydrolysis were titrated to neutralization with 50 mM NaOH in presence of thymolphtalein as indicator. A blank was prepared for each sample where the enzyme was inactivated by heating at 95°c for 15 min. One unite of lipase activity was expressed a micro equivalents of fatty acid released from a triglyceride in 30 min at pH 7.0 at 20°c[21].

Effect of temperature on lipase activity

Lipolytic activity of the partial purified enzyme was determined after 1 hr. of incubation at temperatures 25, 35, 45, 55, 65, 75, 85 ℃, the activity was measured by using Tween 20 as a substrate and the reaction mixture was incubated at the previous temperatures [22].

Effect of pH on lipase activity

The effect of pH on the activity of partial purified lipase was studied at various pH in the range of (3-10) using Tween 20 as a substrate. Buffer solutions (20 mM) of different pH values were used which includes acetate buffer (3,4,5,6), phosphate buffer (7,8,9,10).

The partial purified enzyme was incubated for 1 h. in buffers with varying pH values at 30°C and the lipolytic activity was measured under standard conditions [22].

Effect of some metal ions on lipase activity

For determining the effect of metal ions on lipase activity, the enzyme were per-incubated with 1 mM of metal ions for 1 h. at 30 $^{\circ}$ C.The chloride salts of metal ions tested were $Ca^{++,}$ Na^{+} and Zn^{++} [21].

Results and Disscussion

Atotal number of *A.baumannii* that isolated during the study period was 10(16.6%), out of 60 collected different clinical specimens (4) were isolated from the blood samples, (3) were isolated from pus samples and (3) were isolated from urine samples Table (2). The isolation percentage of *A.baumannii* in recent study nearly to that obtained by Shareek *et al.*[23], and to that obtained by Andreza *et al.*[13].

Table 2: Number of *A. baumannii* isolates from different clinical samples

Clinical Samples	Number of isolates
Blood n=20	4
Pus n=20	3
Urine n=20	3
Total number =60	10(16.6%)

Colonies of pure culture of the above isolates were chacterized by using morphological and biochemical charcteristics Table(3).

Table 3:The morphological and biochemical charcteristics of *A. baumannii*

Test	Result
Morphology	coccobacilli
Motility	non-motile
Growth on MacConkey agar	Slightly pink colonies
Nitrate reduction	positive
Hemolysis	Non-hemolytic
Catalase	positive
Oxidase	negative
Acid from :	
Glucose	positive
Xylose	positive
Galactose	positive
Mannose	positive
Rhamnose	positive
Lactose	positive
Maltose	positive

The ten isolates were coccobacilli, non-motile, catalase positive, oxidase negative, produce acid from (glucose, xylose, galactose, mannose, rhamnose, lactose, maltose), developing colorless to slightly pink colonies on MacConkey agar, non hemolytic on blood agar, negative for nitrate reduction test, in addition, the identification was confirmed by API 20 E system, the isolates of *A.baumannii* acquired the number (0204042) according to the results obtained from the API 20 E system stripFigure 1.

Figure1 :The result of API20E system for *A. baumannii*

The current results of morphological, biochemical test and API 20E similar to that recorded by Xu *etal.* [24], and by Paul *etal*[19]. In the past decade, *Acinetobacter baumannii* has emerged as amajor nosocomial pathogen in many parts of the world, resulting in devastating outcomes in terms of mortality and mobidity [18].

Table (4) showed the results of antibiotics susceptibility test of *A.baumannii* , all isolates of *A.baumannii* were highly resistant to Ceftriaxon, Colistin, Piperacillin, Co-trimoxazol, Tertracycline, Carbenicillin, Amoxicillin, Penicillin G, Gentamicin, Ceftazidim, wherease all of them, were sensitive to Imipenem, Ciprofloxacin, Meropenem, Amikacin, and Cefotaxime Figure 2.

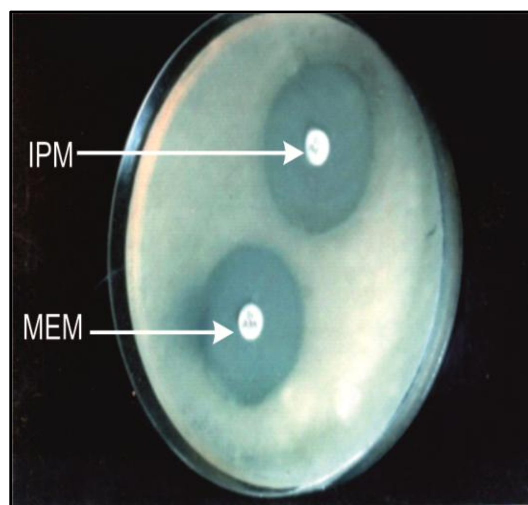

Figure 2:The sensitivity of *A.baumannii* to and Imipenem (10 mcg) IPM and Meropenem (10mcg) MEM

Table 4: The Antibiotics suscebtibility patterns of ten isolates of *A. baumannii*

Antibiotics	Symbol	Resistance%	Sensitivity%
Imipenem (10 mcg)	IPM	-	10(100%)
Ciprofloxacin (5mcg)	CIP	-	10(100%)
Meropenem (10mcg)	MEM	-	10(100%)
Penicillin G(10mcg)	P	2(20%)	8(80%)
Ceftriaxone (30mcg)	CRO	10(100%)	-
Colistin (10 mcg)	CL	10(100%)	-
Cephotaxime (30mcg)	CTX	3(30%)	7(70%)
Piperacillin (100mcg)	PRL	10(100%)	-
Gentamycin (10mcg)	CN	6(60%)	4(40%)
Tetracyclin (30 mcg)	TE	10(100%)	-
Amikacin (30 mcg)	AN	-	10(100%)
Amoxicillin (25mcg)	AMX	10(100%)	-
Ceftazidim (30 mcg)	CAZ	8(80%)	2(20%)
Co-trimoxazol (25mcg)	SXT	10(100%)	-
Carbencillin (100mcg)	PY	10(100%)	-

There are recent reports of increasing resistance to these important drugs, documented all over the world; Prakasam *et al*., reported high level of resistance to Piperacillin, Ceftazidim, Gentamicin ,and Co-trimoxazol[18].

Shareek *etal*., reported that *A. baumannii* were sensitive to Meropenem, Imipenem, Cefotaxime, Amikacin and Ciprofloxacin [23], other reports indicate that the resistance rate for Meropenem, Imipenem, Amikacin and Ciprofloxacin were relatively low, however they were almost more than 30 % [24].

Awide spectrum of antimicrobial resistance mechanisms is exhibited by *A. baumannii*, a part from its intrinsic resistance mainly due to low permeability of the outer membrane to certain antibiotics,production of β lactamases , mutations in antibiotic targets as well as constitutive expression of certain efflux pumps[25].

Avast number of *A. baumannii* lipases have a wide range of potential applications in the hydrolysis, esterification and transesterification of triglycerides [2]. lipase production by *A. baumannii* was confirmed on Rhodamine B plates containing olive oil, the colonies showed orange fluorescence colour when exposed to U.V. light, this is due to the formation of complex between cationic Rhodamine B and the uranyl fatty acid ion [21], the results obtianed from Rhodamine B agar indicated that only one isolate which obtained from pus was strong producer of lipase enzyme.

The above results were also recorded by many researchers like Saeed *etal.*[1], Iftkhar *etal.*[20], and Jagtap *etal.*[26].

The enzyme was partialy purified using ammonium sulphate precepitation method, the maximum total activity, specific activity, yielded (14.3 U/ml, 18.8 U/mg , and 88.2%) were detected at 30% ammonium sulphate respectively ,wherease by increasing the ammonium sulphate concenteration to 80% a slight decrease in total activity was obtianed Table(5).

Table 5: Partial purification of lipase from *A. baumannii* by using ammonium sulphate

Stage	Total activity (U/ml)	Specific activity (U/mg)	Yield %	Purifica - tion factor
Culture filtrate	17.2	4.2	100.0	1.0
30%	14.3	18.8	88.2	4.5
80%	13.8	18.1	84.7	4.3

Some authors recorded that lipase activity increased at 30 % ammonium sulphate than in 80%, this may be due to the fact that ammonium sulphate at high concenterations could result in drop in pH and consequently a loss in enzyme activiy [26,27]. The optimum production temperature for lipase production by *A. baumannii* was at 35°C, no production was detected at 65°C, 75 °C and 85 °C as shown in Figure 3.

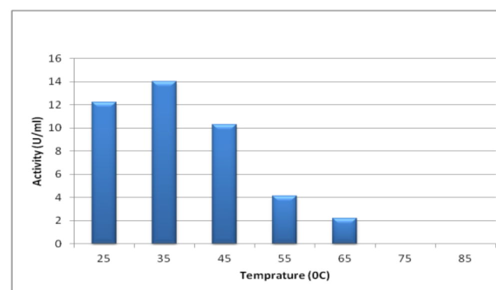

Figure 3: effect of various temperature on lipase activity

Hasan *etal*., recorded that the optimum temperature for lipase production by *Acinetobacter spp.* was 40 °C [28], while Gupta *etal*., recorded that the optimum temperature for lipas production by *Acinetobacter spp.* was 25°C , the bacterial lipases generally have optimal temperature range of 30- 60°C, however reports exist on bacterial lipases with optimal temperature in both lower and higher ranges [2]. The pH range of *A. baumannii* lipase was detectable over a wide range between (3-10) wih an optimum pH value at 7 and no lipase production was detected at acidic pH (3.0, 4.0 ,5.0, 6.0) and exreme alkaline conditions (pH 10.0) Figure 4.

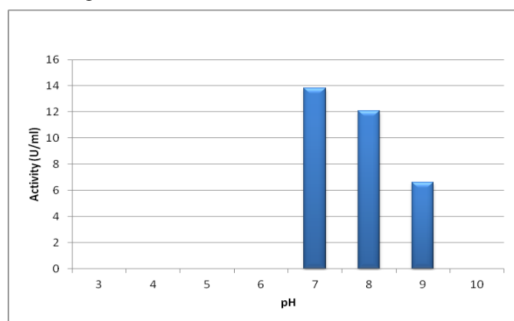

Figure 4: Effect of various pH on lipase activity

The initial pH of the growth medium is important for lipase production, largely, bacteria prefer pH around 7.0 for best growth and lipase production [3].

The pH results are in accordance with Huang *etal.*[29], and Salah *etal.*[30], the enzyme was stable at pH range (7-8) and also retained 90% of its activity as reported by Kasana *etal*., [31].

The effect of some metal ions on the activities of *A. baumannii* lipase was also investigated, it was found that NaCl, $CaCl_2$ enhance the lipolytic activity of partial purified lipase and inhibited by Zn^{++}(Table 6). The observation that lipase activity was significantly enhanced in the presence of these metal ions probably reflects the abilityof these salts to react with free fatty acids.

Sharma *etal*., reported that lipase activity was enhanced by the presence of Na^+ and Ca^{++} [32]. similar kind of work has also reported by Kumer *etal.*[33], and by Patil *etal.*[6] wherease Park *et al*., recorded that the Zn^{++} has inhibitory action on *A. baumannii* lipase [34] but other study reported that lipase activity is slightly inhibited by Zn^{++}[1].

Conclusion

Bacteria constitute naturally immobilized lipases which have high catalytic power and stability. *A.baumannii* exhibited the ability to produce lipase enzyme. Various physicochemical parameters were studied to determine the optimum conditions for its lipase production, the optimum pH for this enzyme was 7.0, the optimum temperature was 35°C, enhanced by Ca^{++} and Na^+ ions ,while inhibited by Zn^{++}ions. The partial purification of lipase by using ammonium sulphate at 30% saturation showed the high activity.

Further studies are recommended on the other strains of *Acinetobacter* sp, for much more lipolytic activity and on the other conditions affected the producing of this enzyme .

References

1. Saeed H., Zaghoul T., Khalil A., Abdelbaeth M.,. Purification and characterization of two extracellular lipases from *Pseudomonas aeroginosa* Ps-x, polish J. Microbiol., 2005, 54(3):243.
2. Gupta R., Gupta N., Rathi P.,. Bacterial lipases: an overview of production, purification and biochemical properties, Appl. Microbiol. Biotechnol, (2004), 64: 763–781.
3. Jianrong L., Yu D., Haiming J, Lili C., Xin Z., Yunming T.,. Production, purification and characterization of lipase from *Serratia sp.SL-11,* J. Biotechnol.,2011 ,29 : 120-121.
4. Chouhan M., Dawande A.,.Partial purification, characterization of lipase produced from *P. aeruginosa*, Asiatic J. Biotechnol. Res. 2010; 01: 29-34.re
5. Bonala K., Mangamoori L. Screening, isolation and identification of extracellular lipase producing bacteria from oil mill waste, J. Chem. Bio. Phy. Sci. Sec. B. 2012, 2(2):814-819.
6. Patil K., Chopda M., Mahajan R.,. Lipase biodiversity, Indian J. Sci. and Technol.2011, 4 (8) :972-977.
7. Breiji A., and Anastasia D., Towards an explanation for the success of *Acinetobacter baumannii* in the human host, Lieden university repository, 2012 : (11-12).
8. Oberoi A., Aggarwal A., Lal M.,. A decade of an under estimated nosocomial pathogen-*Acinetobacter* in a tertiary care hospital in Punjab. JK science, 2009, 11: 24-26.
9. Jawetz, Melinck and Adelbergs, Medical Microbiology, 24th ed., McGraw –Hill company, 2007, 266-267.

10. Nemec A., Krizova L., Maixnerova M., van der Reijden T., Deschaght P., Passet V., Vaneechoutte M.,Brisse S., Dijkshoorn L., Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittiisp*. nov.(formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). Res Microbiol0, 2011, 162: 393-404.

11. Anbu P., Ju Noh M., Hye Kim D., SeokSeo J., Ki Hur B., Ho Min K., Screening and optimization of extracellular lipases by Acinetobacter species isolated from oil-contaminated soil in South Korea,Afr.J. Biotechnol., 2011,10(20): 4147-4156.

12. Chen H., Chen T., Lai C., Fung C., Wong W., Yu KW., Liu C. ,. Predictors of mortality in Acinetobacter baumannii bacteremia. J. Microbiol. Immunol.Infect., 2005, 38: 127-136.

13. Andreza A., Kuchenbecker R., Pilger K., Pagano M., Afonso L. ,. High endemic levels of multidrug-resistant Acinetobacter baumannii among hospitals in southern Brazil, Amer. J. Infec. Control, (2012), 40: 108-12.

14. Van Looveren M., Goossens H. ,.Antimicrobial resistance of Acinetobacter spp. in Europe. Clin.Microbiol. Infect,2004, 10: 684-704.

15. Venezia S., Ben-Ami R., Carmeli Y. ,. Update on Pseudomonas aeruginosa and Acinetobacter baumannii infections in the healthcare setting. Curr.Opin. Infect. Dis.,2005, 18: 306-313.

16. Livermore D., Woodford N. ,.Carbapenemases: a problem in waiting? Curr.Opin.Microbiol, 2000, 3:489-495.

17. Peleg AY., Seifert H., Paterson DL. ,. Acinetobacter baumannii: emergence of a successful pathogen. Clin. Microbiol. Rev. ,2008, 21: 538-582.

18. Prakasam G., Geethapriya S., Jayakeerthana K., Ramesh S.,. Detection of certain virulence attributes and antimicrobial resistance pattern among clinical isolates of Acinetobacter baumannii, International J. Pharma. Bio Sciences, 2011, 2: 502-503.

19. Paul G., Janet E., and Duben E.,. Laboratory diagnosis of infectious diseases, Lippncott Williams and Wilkins, 2008, 169-170.

20. Iftikhar T., Niazm M., Jabeen R., Ulhaq I.,. Purification and characterization of extracellular lipases , Pak. J. Bot., 2011, 43(3): 1541-1543.

21. Kukreja V., Bera M.,. Lipase from Pseudomonas aeroginosa MTCC 2488: Partial purification, characterization and calcium dependent thermostability, Indian J. Biotachnol., 2005, (4):222-226.

22. Rajeswari T., Palaniswamy C., Venil C., Nathia K., Joyruth P., Production of partial purification and characterization of lipase from Aspergillus flavus KUF 108. 2010. Pak. J. Sci. Ind. Res., 53 (5): 258-262.

23. Shareek, P.S., Sureshkumar D, Ramgopalakrishnan, Ramasubramanian, Ghafur K., Thirunarayanan M., Antibiotic Sensitivity Pattern of Blood Isolates of Acinetobacter Species in a Tertiary Care Hospital: A Retrospective Analysis, 2012, Am. J. Infect. Dis., 8 (1): 65-69.

24. Xu J., Wu H., Li J, Zhou Q.,. Antimicrobial resistance surveillance of Acinetobacter baumannii isolated from teaching hospitals, International conference on future information technology. 2012,(14):379-380.

25. Allen DM., Hartman B., Principles and Practice of infectious Diseases, 6th ed. Churchill Livingstone, Philadeliphia, 2005, 2632 -2636.

26. Jagtap S., Gore S., Yavankar S., Pardesi K.,Chopade B.,. Optimization of medium for lipase production by Acinetobacter haemolyticus from healthy human skin. Indian J. Exp Biol.,2010, 48: 936-941.

27. Yoon M., Shin P., Han Y., Lee S., Park J., Cheong C.,.Isolation of an Acinetobacter juniiSY-01 strain producing an extracellular lipase nantioselectively hydrolyzing it raconazole precursor and some properties of the lipase. J. Microbiol. Biotechnol.2004,14: 97-104.

28. Hasan F., Shah A., Hameed A. ,. Methods for detection and characterization of lipases: A comprehensive review, Biotechnol. Advances, 2009, 27 : 783.

29. Huang Y., Locy R., Weete J.,.Purification and Characterization of an Extracellular Lipase from Geotrichum marinum. Lipids.2004, 39: 251-253.

30. Salah B., Rajeswari T., Noushida E., Sethupalan D., Venil C., Production of lipase enzyme from lactobacillus spp. and its application in the degradation of meat, World Appl.Sci. J., 2011, 12(10) :1799-1780.

31. Kasana R., Kaur B, Yadav S, Isolation and identification of a psychrotrophic Acinetobacter sp. CR9 and characterization

of its alkaline lipase. J. Basic Microbiol.,2008, 48(3):207-12.

32. Sharma R, Chisti Y, Banerjee U, Production, purification, characterization, and applications of lipases, J.biotechnol., 2001, 19 (2) : 627- 662.

33. KumerH., Kim SH, Lee YS, Lee SC, Zhou Y, Kim CM, et al. Gene cloning, purification, and characterization of a cold-adapted lipase produced by Acinetobacter baumannii. J Microbiol.Biotechnol 2009;19(2):128–35.

34. Park I., Kim S., Lee Y., Lee S., Zhou Y., Kim C., AhnS.,. Gene cloning, purification and characterization of a cold adapted lipase produced by Acinetobacter baumannii BD5. J.Microbiol. Biotechnol.2009, 19: 128-135.