Characterization of β-lactamase from local isolate of *Staphylococcus aureus*[+]

توصيف لأنزيم البيتالاكتاميز المعزول من عزلة محلية لجرثومة المكورات العنقودية الذهبية

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Abstract

The Characterization of β-lactamase that isolated from local isolate of *Staphylococcus aureus* has been studied, the suitable temperature for enzyme activity ranged between (25 to 45)°C with an optimal temperature at (25, 30, 35)°C. The enzyme was retained 83% of its activity after incubation for 10 min. at 45°C, while it has been lost 48% of its activity after 60 min. of incubation. Also, the enzyme was active at acid reactions between (4.5 to 7.5) with an optimal pH (6, 6.5, 7). The enzyme activity was not inhibited by $AgNO_3$, $CaCl_2$, KCl, NaCl, and EDTA, while 2-mercaptoethanol (1mM) inhibited 98% of the enzyme activity and (30µg/ml) of Augmentin inhibited 73% of enzyme activity.

الخلاصة

تم دراسة خصائص انزيم البيتالاكتاميز المعزول من عزلة *Staphylococcus aureus* المحلية وتم تحديد درجة الحرارة الملائمة للأنزيم وباستخدام مدى حراري تراوح بين (٢٥-٤٥)°م، وكانت الدرجة الحرارية المثلى هي (٢٥، ٣٠، ٣٥)°م. احتفظ الأنزيم بنسبة ٨٣% من فعاليته بعد حضنه لمدة ١٠ دقائق في درجة حرارة ٤٥°م، بينما فقد ٤٨% من فعاليته بعد فترة حضن لمدة ساعة كاملة. كانت الدالة الحامضية المثلى لفعالية الأنزيم هي (٦، ٦،٥ ،٧) إذ تم متابعة فعالية الأنزيم بمدى من الدالة الحامضية تراوح بين ٤،٥ إلى ٧،٥. لم تبدي المواد $AgNO_3$، $CaCl_2$، KCl، NaCl وEDTA عند معاملتها مع الأنزيم أي تأثير على الفعالية الأنزيمية، إلا أن (١مايكرومولاري) من مادة 2-mercaptoethanol ثبط فعالية الأنزيم وبنسبة ٩٨% وكذلك (٣٠مايكروغرام/مل) من مادة Augmentin استطاعت تثبيط فعالية الأنزيم وبنسبة ٧٣%.

Introduction

Staph. aureus is one of the major resistant pathogens, found on the mucous membranes and the skin of about third of the population around the world, it is extremely adaptable to antibiotic pressure. It was the first bacterium in which penicillin resistance was found [1], most strains of *Staph. aureus* are now resistant to penicillins, this is because it can make a β-lactam hydrolyzing enzymes called β-lactamases that degraded penicillins, destroying its antibacterial activity, they probably also, serve other cellular maintenance roles involving the bacterial cell wall [2]. β-lactamases are enzymes (penicillin amido-β-lactam-hydrolyse,EC

[+]**Date received 26/1/2009 Date Accepted 1 / 11 / 2009**

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3.5.2.6.) produced by the great majority of clinically important G+v and G-v bacteria, and are responsible for their resistance to β-lactam antibiotics like penicillins, cephalosporins, and carbapenems, these antibiotics have a common element in their molecular structure: four-carbon atoms ring known as a β-lactam ring [3,4], these enzymes orient the β-lactam antibiotics in a precise manner in its active site to facilitate the breaking of the C-N bond in the four membered β-lactam ring, then destroying the antibiotics and produce antibacterial inactive products [5,4]. *Staph. aureus* resistance to penicillins is partly mediated by these an inducible enzymes in which bacterium secrete it into the surrounding environment. Four variants of *Staph. aureus* β-lactamases with slightly different chemical and enzymatic properties have been found in different isolates of *Staph. aureus,* and can be distinguished by serotype [6,7], and kinetic attributes [8, 9,10].

Materials and methods

β-lactamase enzyme was isolated from local isolate of *Staph. aureus* that isolated from nasopharyngeal region of healthy individuals, the enzyme was obtained from [11].

Assay of β-lactamase.

The enzyme activity was determined by a micro-iodometric assay according to [12,13].

The Stability of enzyme

The determination of enzyme stability employed according to [14], the enzyme solution was incubated at 45°C for different time periods (10, 20, 30, 40, 50, 60, 120)min., and cooled directly in ice-bath, The remaining enzyme activity was determined to non treated enzyme and the relation was drown between different time periods and the remaining activity percentage.

The Optimum temperature of enzyme

The determination of optimal temperature for enzyme activity was employed according to [15], the enzyme activity determined in a range of temperature degrees between (25-60)°C in the scale of 5 degree. The relation was drown between temperature and enzyme activity.

The Heat stability of enzyme

1ml of enzyme solution was dispensed in test tubes and incubated in water-bath with different temperature degrees ranged from 25°C to 90°C for 15min., then the test tubes cooled directly in ice-bath, and the remaining activity was determined according to non treated enzyme [14]. The relation was drown between temperature degrees and the percentage of remaining enzyme activity.

Acid reactions of the enzyme

solutions:-

1. Sodium acetate buffer (0.05M) to adjusted pH at (4.5, 5.5, 6).
2. Sodium phosphate buffer (0.05M) to adjusted pH at (6.5, 7, 7.5).
3. Tris-buffer (0.05M) to adjusted pH at (8, 8.5, 9, 9.5, 10).

Solutions for substrate (penicillin-G) was employed in final concentration (0.0089g/10ml) by using Sodium acetate buffer (0.05M) for pH (4.5, 5.5, 6), sodium phosphate buffer (0.05M) for pH (6.5, 7, 7.5), and Tris-buffer (0.05M) for pH (8, 8.5, 9, 9.5, 10). Enzyme activity was determined, the relation was drown between pH values and enzyme activity [16].

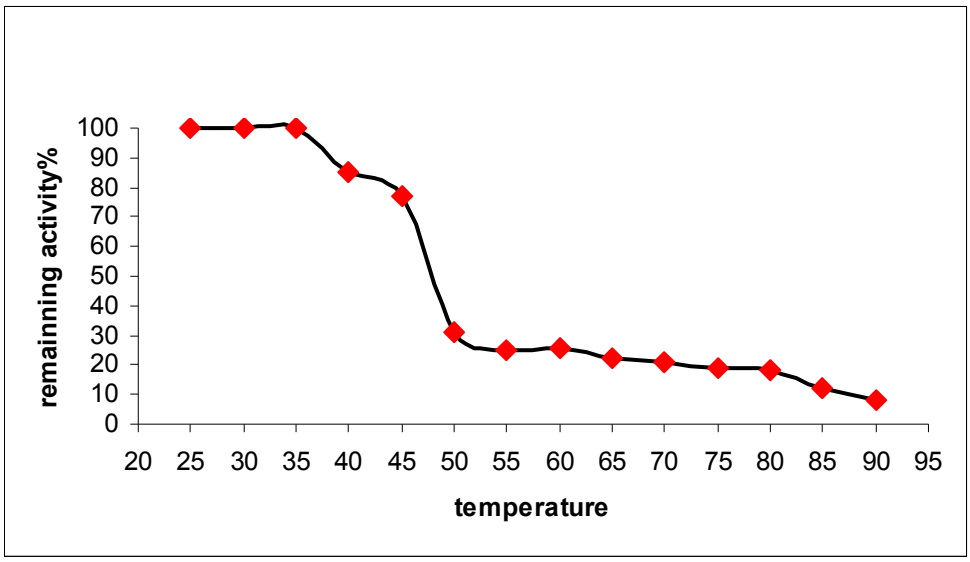
Enzyme was incubated with different ranges of pH at 30°C for 15min. This performed by mixing equaled volumes of enzyme and buffer solutions with different pH ranged from 4.5 to 10, then cooled in ice-bath, the remaining enzyme activity was determined.

Effect of chemical compounds and mineral ions on enzyme activity.

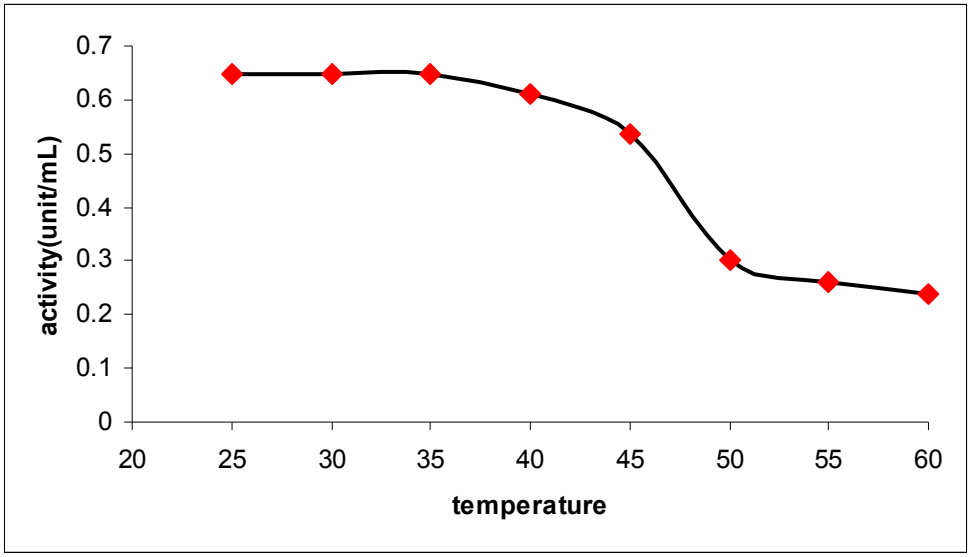
The enzyme solution was mixed with $CaCl_2$, KCl, NaCl, 2-mercaptoethanol and EDTA solutions at a final concentrations (1, 2 Mm), while the enzyme solution was mixed with solutions of $AgNO_3$ and Augmentin in a final concentrations of (0.1, 0.2 mM) and (20, 30 µg/ml) respectively, the mixture was incubated at 35°C for 10 min. The remaining enzyme activity was determined [17].

Results and Discussion

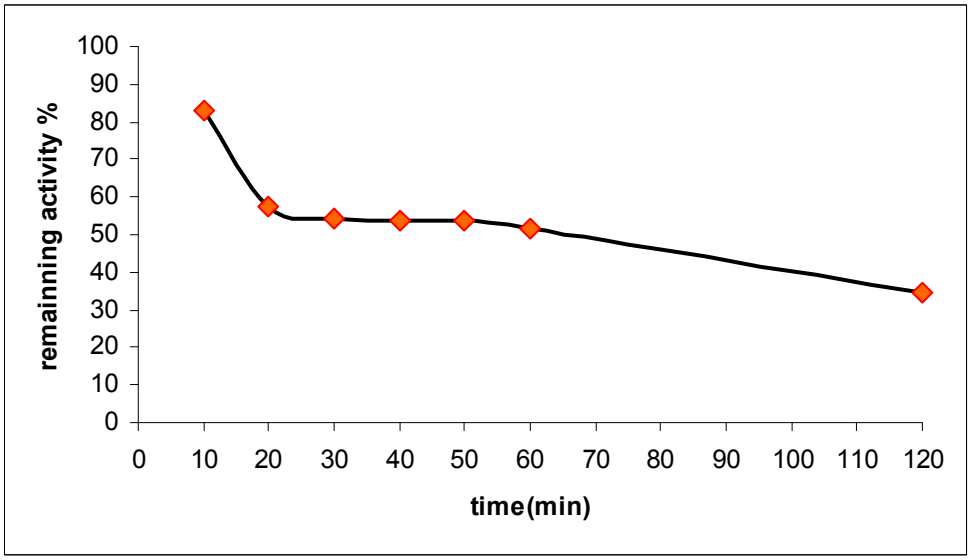
In the present study, the enzyme was incubated at a different temperature ranged from 25°C to 90°C for 15min., the enzyme was retained its optimal activity at the temperatures between (25°C- 35°C), then the activity was decreased with further increasing of temperature, and lost 15% of its activity at 40°C, while 31% of the enzyme remained active at 50°C as showed in Fig(1). To determine the optimal temperature of enzyme activity, catalytic enzyme reaction was employed in a temperature ranged between 25°C to 60°C, and the results were revealed that the optimal temperature was (25, 30, 35°C) as showed in Fig(2). The results of enzyme stability at 45°C for different time periods (10min. – 120min.) was showed that the 17% of enzyme activity was lost after 10min. of incubation, while the 52% remained active after 60min. of incubation as showed in Fig(3). However, only few studies on β-lactamase were related to influence of incubation temperature [18], and a high temperature molecular dynamics of unfolding intermediates [19].



Fig(1). Enzyme stability at different temperature degrees

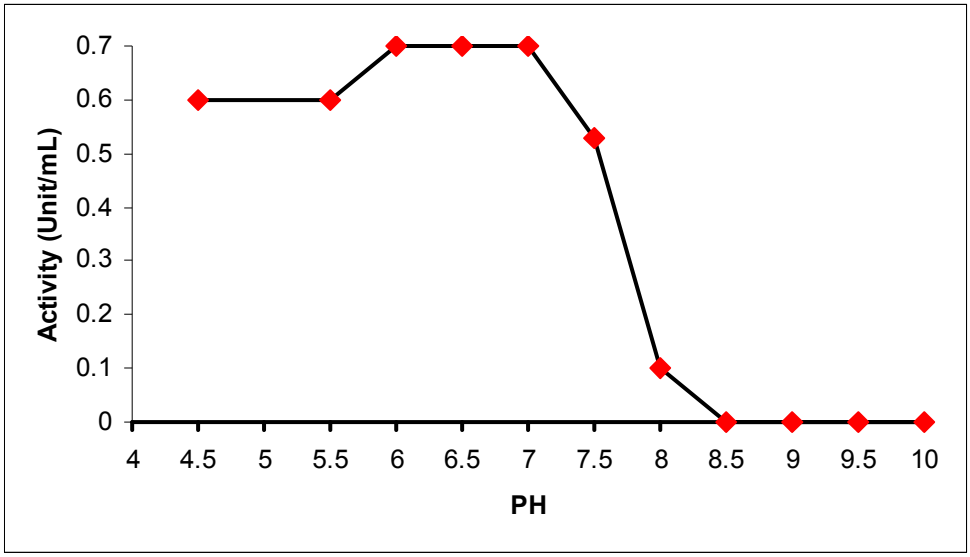


Fig(2). Effect of different temperature degrees on enzyme activity

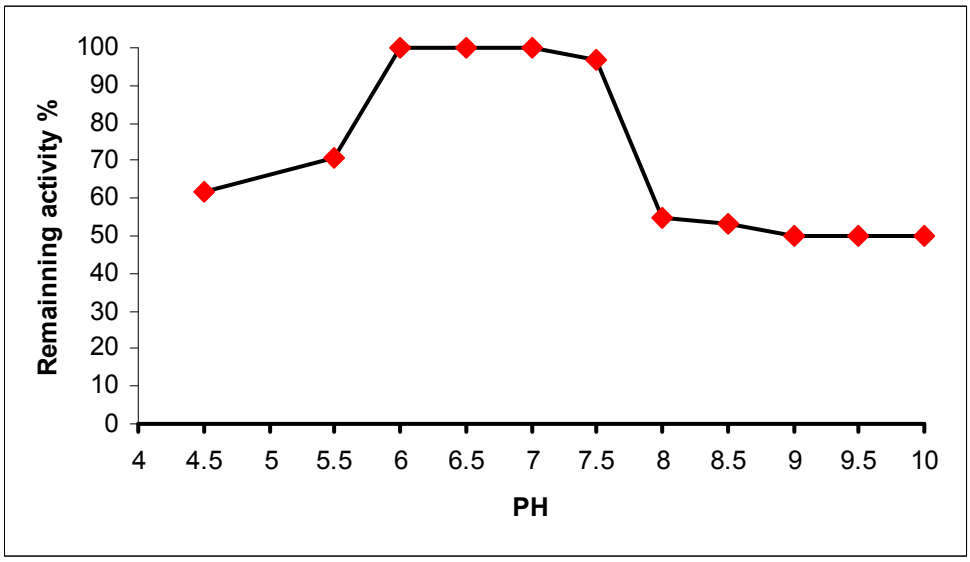


Fig(3). Enzyme stability at 45°C for different time periods.

The effect of pH on enzyme activity was studied, and the results were revealed that the optimal pH to remain the enzyme activity were (6, 6.5, 7) Fig(4)., while the activity was decreased at extreme value of pH especially at (8 – 10). Enzyme was stable at a range of pH between (6 – 7.5), and the optimal pH for enzyme stability was (6, 6.5, 7) as showed in Fig(5). However, these results have many similarities in common with the β–lactamase isolated from *Pseudomonas cepacia* 249 [20].



Fig(4). Enzyme activity at different pH degrees



Fig(5). Enzyme stability when incubated with different pH degrees (4.5-10).

Table(1), showed that the enzyme was lost its whole activity after treated with (2mM) of 2-mercaptoethanol (a reducing agent which breaking a disulfide bond). This result was indicated that the enzyme contained a disulfide bond, therefore, β-lactamase in present study perhaps classified into A carbapenem - hydrolysing enzymes, these enzymes have a disulfide bridge which creates a new covalent bond and thus significantly modifies the active site geometry [21, 22]. The results in Table (1) was showed that the Augmentin (a combination of Amoxycillin and Clavulanic acid) decreased the activity of enzyme to (45 and 27) % at the concentrations of (20 and 30) µg/ml respectively, these results agree with results of Catherall *et al.*, who showed that Amoxycillin/ Clavulanic acid was more effective than Amoxycillin alone against endocarditis caused by β-lactamase producing strains of *Staph. aureus* [23]. The enzyme activity in present study was not effected by, $AgNO_3$, $CaCl_2$, KCl, NaCl,

and EDTA as cleared in Table(1). The simple rise or decline from 100% remaining activity as showed in Table below was meaning that there was no effect of chemical compounds or mineral ions on enzyme activity [15].

Table(1). Effect of some chemical compounds and mineral ions on enzyme activity

Materials	Concentration	Remaining activity %
Control	-	100
$AgNO_3$	0.1mM 0.2mM	102 99
$CaCl_2$	1mM 2mM	103 107
KCl	1mM 2mM	101 97
NaCl	1mM 2mM	102 102
2-mercaptoethanol	1mM 2mM	2 0
EDTA	1mM 2mM	97 97
Augmentin	20µg/ml 30µg/ml	45 27

Conclusions

The suitable temperature for β-lactamase activity was ranged between (25 to 45) °C, with an optimal temperature at (25, 30, 35) °C. , also the enzyme was active at acid reaction between (4.5 to 7.5) with an optimal pH (6, 6.5, 7).

The 2- mercaptoethanol (1mM) was inhibited 98% of the enzyme activity and 30µg/ml of Augmentin was inhibited 73% of the enzyme activity. $AgNO_3$, $CaCl_2$, KCl, NaCl, and EDTA have no effect on enzyme activity.

Recommendation

We recommended to continue the studies that related to β-lactamases by using other inhibitor substances, especially that don't have effects on human beings, and use it to reduce the effect of these enzyme

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