Research Article

Purification and characterization of protease and lipase from *Pseudomonas aeruginosa* isolated from some wound and burn infection

Yazi Abdullah Jassim, Shaemaa Muhi Hasson Al-Amery*

ABSTRACT

Background: *Pseudomonas aeruginosa* is a type of bacteria that causes many diseases, especially hospital infections (nosocomial infections) because of its wide spread in the environment, especially the hospital environment. **Methodology:** Twenty isolates of *P. aeruginosa* were identified. **Results:** The isolates were 12 isolates from burn infections and 8 isolates from wound infections. The sensitivity of *P. aeruginosa* isolates was tested against (6) traditional antibiotics. *P. aeruginosa* was (100%) resistance to Ampicillin, 94.5% resistance to Cefixime, (80%) to Ceftazidime, (38.9%) to Tobramycin, 22.9% to Norfloxacillin and 3.4% to Ciprofloxacin . By observing hydrolysis zone around the colony of bacteria that grow on Peptone tween agar plate, we confirmed the lipolytic ability and produce lipase enzyme. Their abilities for production of protease enzyme was tested on skim milk agar by appeared clear zone round the colony. The optimum conditions of enzymes production were studied such as (PH, temperature) which show that 40°C , pH (8) and incubating to 48 hr. **Conclusion:** The enzyme Activity was decremented in these optimum conditions and The activity of the enzyme has increased when treated with Ca ions, which shows that Ca^{++} plays a role in the stimulation and stability of the enzyme, and did not inhibit the activity of the enzyme with the presence of reduced agents such as cysteine, while decrease the enzyme's effectiveness of the factors EDT.

KEY WORDS: Nosocomial infection, *P. aeruginoas*, Proteolytic enzyme

INTRODUCTION

Pseudomonas aeruginosa is a type of bacteria that cause many diseases, especially hospital infections (nosocomial infections) due to its widespread in the environment, especially the hospital environment. These bacteria ranked second among the species that cause clinical diseases in the United Kingdom, especially cases of pneumonia in the intensive care unit (4.11%) in Latin America and Asia. In Europe, the infection rate was 3.9% while in the UK was 7.8% and in Canada was 6.8%.[1] *P. aeruginosa* caused respiratory diseases, especially cystic fibrosis. The incidence of the disease was 60–90% at the Copenhagen Hospital in Denmark, another study found that 80% of cystic fibrosis was the causative bacteria,[2,3] and in a local study in Iraq,[4] the highest incidence of bacterial infection *P. aeruginosa*. *P. aeruginosa* causes other diseases, including infections of burns, eye infections, skin infections, bacteremia, and infections in people with cancer, with HIV and AID.[5] Moreover, it also caused inflammation of urinary tract infections, chronic middle and outer ear infections, eye inflammation, lower respiratory tract infections, gastrointestinal inflammation, pneumonia, septicemia, endocarditis, nervous central infection system, and joint and bone infections (gastrointestinal infections) as well as soft tissue and skin lesions.[6] Protease and lipase enzymes are abundant in nature and in different sources. Microorganisms such as bacteria, fungi, and yeast are the most important sources of proteins so that their production can be controlled through easy control of the environment, in which they grow and the ease of developing their production through genetic engineering techniques. Rapid growth in the low-income diets[7] as well as some microbial enzymes is more likely to be derived from plants and animals than their counterparts because their production is easier and more manageable.[8] *P. aeruginosa* possesses many of virulent factors such as toxins and enzymes that have an effect on their pathogenesis. The enzymes includes: lipase , Hemolysin as well as protease,[9] the

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Department of Biology, College of Science, University of Babylon, Babylon Province, Iraq

***Corresponding author:** Shaemaa Muhi Hasson Al-Amery, Department of Biology, College of Science, University of Babylon, Babylon Province, Iraq. E-mail: shaemaamuhi@yahoo.com

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objective of this study was to isolate and diagnose *P. aeruginosa* bacteria from different disease cases and to study the sensitivity of these isolates to various antibiotics and to detect and characterize the protease and lipase produced by this bacterium.

MATERIALS AND METHODS

Isolation and Diagnosis of Bacteria

The samples collected under the supervision of the medical specialist were collected from different medical conditions including 40 wound swabs and 55 burn swabs, and the samples were cultured on the Blood and MacConkey agar. The biochemical tests were carried out according to the standard methods followed[10] to diagnose the bacteria.

Test the Sensitivity Test of *P. aeruginosa* to Traditional Antibiotics

The sensitivity test of *P. aeruginosa* was performed using the following antibiotics as clear in Table 1.

Screening of Enzymes Production

Protease enzyme

The bacteria were grown on skim milk agar for 24 h at 37°C, and the diameter of the decomposition zone was measured and Ps3 isolates were selected, which gave the widest diameter of the decomposition zone and were isolated from burns.[11]

Lipase enzyme

The bacteria were grown on peptone Tween 80 agar for 24 h at 37°C and the diameter of the decomposition zone. The Ps6 isolates were selected, which gave the widest diameter of the decomposition zone and were isolated from wound infections.[12]

Determent Optimum Conditions

Optimum temperature

The optimal temperature was studied using different degrees[10,13,14] and after that, the enzymatic activity was estimated.[15]

Optimum pH

A different pH was used between 7 and 10 (after which, the enzymatic efficacy was estimated).[15]

Extract and Purify the Enzyme

The enzymes (lipase and protease) of *P. aeruginosa* bacteria were purified using the cellulose-diethylaminoethyl (DEAE) ion-exchange chromatography.[16]

Study of the Effect of Some Metallic Ions and Reduce Agents in the Activity of the Enzyme

The effect of some metal ions and reduce agent on the efficacy of the enzyme, which included cysteine, sodium azide, $ZnCl_2$, $MgSO_4$, $HgCl_2$, $CaCl_2$, EDTA, and SDS NaCl was studied to detect the enzyme activity.

RESULTS AND DISCUSSION

Isolation of *P. aeruginosa*

Twenty isolates of *P. aeruginosa* bacteria have been diagnosed with standard methods. There were eight isolates of wound inflammation and 12 isolations of burns. Table 2 shows the isolation of *P. aeruginosa* from different disease cases. The bacteria are *P. aeruginosa* of the most common types of bacteria causing hospital injuries (nosocomial infection) has been isolated from various disease cases and especially from post-operative infections. In most studies, it was found that *P. aeruginosa* bacteria are the main causative isolation of wounds and burns.[17]

The Sensitivity of *P. aeruginosa* to Traditional Antibiotics

The sensitivity test was doing against six antibiotics and isolates showed different ratios in antibiotic resistance as shown in Figure 1. Flamm *et al*.[18] showed that the highest resistance of these bacteria was against ceftazidime (76%). The results showed that both norfloxacin and ciprofloxacin showed high efficacy against aeruginosa bacteria. Jobling[19] showed that *P. aeruginosa* isolates isolated from wound infections were sensitive to high levels of ciprofloxacin, cefepime, and amikacin for antimicrobials and imipenem. The increased use of antibiotics randomly increased the appearance of resistance, especially in Gram-negative bacteria. To reduce resistance, the use of antibiotics should be determined.

Screening of Enzymes Production

All *P. aeruginosa* isolates were cultured on the center of skim milk agar and by the diameter of the decomposition around the colonies, it was found that

Table 1: Antibiotics concentration used in sensitivity test

Antibiotic	Concentration
Ampicillin	10 µg
Cefixime	30 µg
Ceftazidime	30 µg
Tobramycin	10 µg
Norfloxacin	10 µg
Ciprofloxacin	5 µg

Table 2: Source of isolating *P. aeruginosa* bacteria from different disease cases

Isolates source	Number of samples	*P. aeruginosa* numbers
Burn infections	42	12
Wound infections	33	8
Total	75	20

P. aeruginosa: Pseudomonas aeruginosa

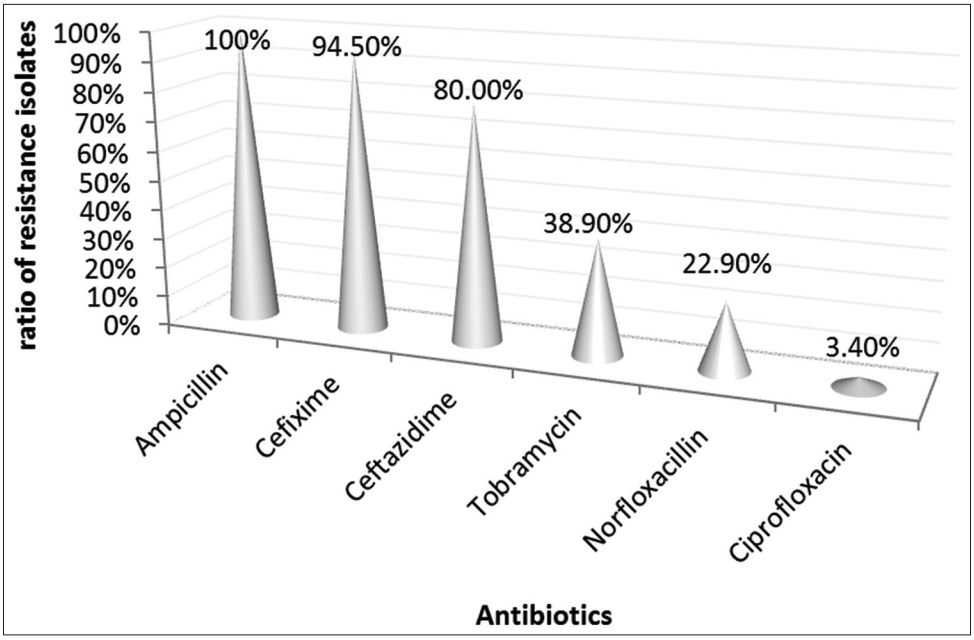

Figure 1: The sensitivity of *Pseudomonas aeruginosa* to traditional antibiotics

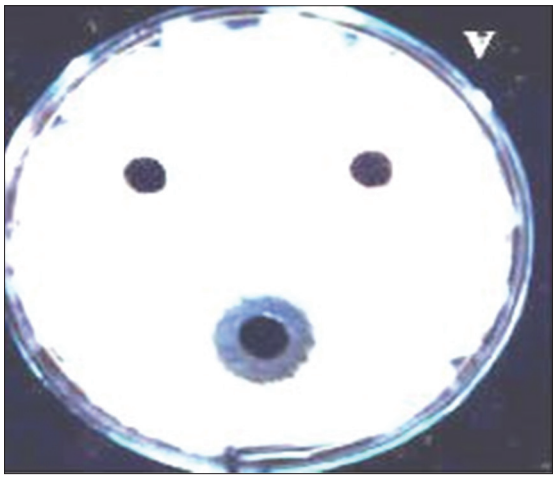

Figure 2: Protease activity

Ps3 isolation was the highest in production, with a diameter reached to 35 mm as shown in Table 2 and Figure 2. While Ps6 was the highest in production of lipase when all *P. aeruginosa* isolates were cultured on peptone tween 80 agar with diameter reached to 29.5 mm as showed in Table 3 and Figure 3.

Optimum Conditions

Optimum temperature

The optimum temperature for protease and lipase production was determined by the isolation of *P. aeruginosa* with values ranging between 10°C and 45°C, and the results showed that the highest productivity at the level of 40°C, as shown in Figure 4, where the qualitative activity 19.5 units/mg and decreased to 9.7 units/mg at 10°C affects the temperature in the production of the protease enzyme and 17.7 units/mg in production of lipase. Through its effect on the solubility of materials in the used plant and its transition, which is reflected in the growth of the microorganism and its production of different enzymes.[20]

High temperature increases the speed of enzymatic reactions due to the increased kinetic energy of the reacting molecules. Thus, the kinetic energy of the enzymes becomes greater than the energy. Breaking the hydrogen bonds that maintain the triglyceride and secondary structure of the enzyme. At this temperature, the enzyme is accompanied by a rapid loss of catalytic effect.[21]

Optimum pH

The effect of pH was studied on the activity of the enzyme produced by *P. aeruginosa* isolation with a pH range of 5–10 with the HCl-Tris solution at a concentration of 2.0 molar. The results are shown in Figure 5 that the optimal pH of the protease enzyme produced from local SP3 is 6.5, (8.6 units/mg), while the efficiency was decreased at pH and acid values, with a specific effectiveness of 5.8 at pH 5.5; the optimal pH of the lipase enzyme produced from local sp6 was 7 (9.2 units/mg). These results are agreement with those reported in studies and research on the enzyme *P. aeruginosa* because the optimal pH of the enzyme is 6.[13,22]

The change in pH will change in the form of proteins, including enzymes, as the transitions of distant peripheral groups change from the binding of the substrate to the enzyme. Often, this leads to the disintegration of the enzyme into protein units and thus loses its activity.[23] On the other hand, the substrate is affected because it contains ionizing groups which affect their association with the enzyme.[24]

Table 3: The enzymatic activity of protease and lipase produced by *Pseudomonas aeruginosa* isolates

Enzyme screening	Diameter of clear zone (mm)																			
	Burn infections												Wound infections							
	Ps1	Ps2	Ps3	Ps4	Ps5	Ps6	Ps7	Ps8	Ps9	Ps10	Ps11	Ps12	Ps1	Ps2	Ps3	Ps4	Ps5	Ps6	Ps7	Ps8
Protease	12	13.6	35	20	25.2	23	22	18.6	19	27	32	21.7	30	23.4	19	28	26	12.6	25.1	16
Lipase	22	18.6	22	24.5	28	24.2	18	13.3	23	23	12	8.9	9.5	23	16.4	8	0	29.5	21	9.4

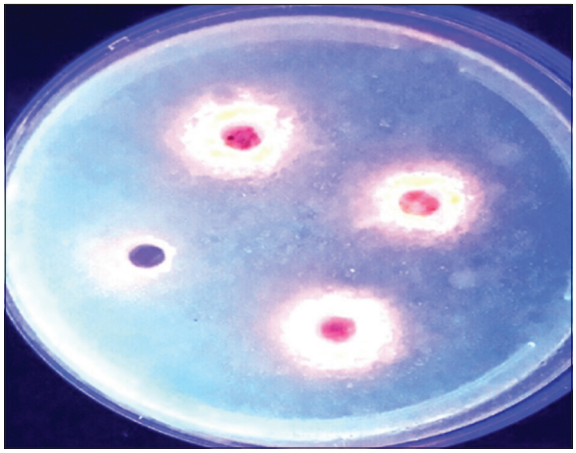

Figure 3: Lipase activity

Extraction and Purification of Protease Enzyme

Aluminum sulfate was used in the deposition to remove a large percentage of water and other proteins to increase the concentration of the enzymes, and the saturation rate was gradually increased to 60%. This percentage of saturation was used by a number of researchers to purify the enzymes from *P. aeruginosa* bacteria.[25] The results of Table 4 showed that after the deposition of aluminum sulfate, an increase in protein concentration and an increase in the specific efficiency to 34.05 units/mg, the number of purification times (1.60), and the enzyme yield (35.99%).

The Cellulose-DEAE Ion-exchange Chromatography

Transfer the protein solution from the deposition process in the cellulose-DEAE column as a second phase. The specific activity was 59.80 units/mg, the number of purification time was 2.87, and the enzyme yield was 36%.

The molecular weight of the purified enzyme (21379) was studied. These results are similar to those of some studies that reported that the molecular weight of the protease enzyme purified from the bacteria itself ranged between 20,000 and 33,000 Dalton.[14]

Extraction and Purification of Lipase Enzyme

The enzyme is purified with ammonium sulfate and with enzyme activity[11] and enzyme yield (40%) as a first step of purification as shown in Table 5.

Study of the Effect of Some Metallic Ions and Reduce Agents in the Activity of the Enzyme

At the incubation of the enzyme with specific concentrations of ions for different metals, their effect on the activity of purified enzymes from *P. aeruginosa* where the results indicated the difference in the effect of ions in the activity of the enzyme, it was observed that the Mg^{2+} and Ca^{2+} ions increased the activity while the K^{+} and Zn^{2+} ions decreased the enzymatic

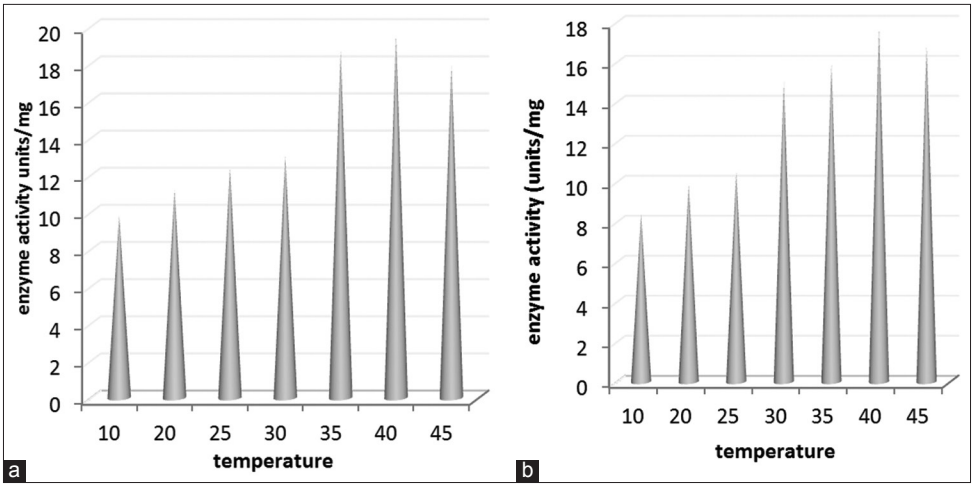

Figure 4: Effect of temperature on enzymes activity. (a) Effect of temperature on protease activity, (b) effect of temperature on lipase activity

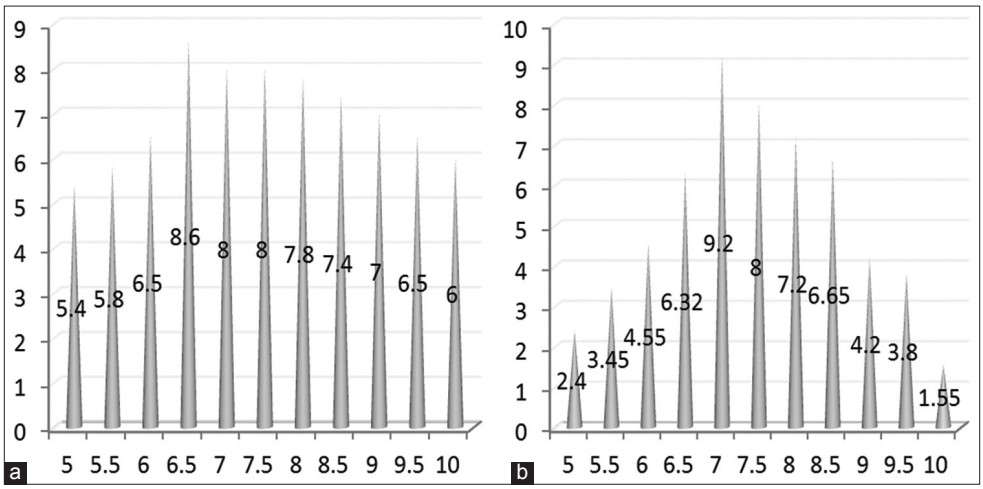

Figure 5: Effect of pH on enzymes activity. (a) Effect of pH on protease activity, (b) effect of pH on lipase activity

Table 4: Extraction and purification of protease enzyme

Step	Volume (ml)	Activity (unit/ml)	Total activity (unit)	Protein concentration (mg/ml)	Specific activity	Purification on fold	Yield %
The extracted enzyme	100	13.33	20.53	0.62	13.33	1	100
Deposition with aluminum sulfate	60	9.1	0.27	41.65	34.05	1.6	35.99
Ion-exchange chromatography DEAE-cellulose	30	15.21	43.26	0.25	59.8	2.87	36

DEAE: Diethylaminoethyl

activity as showed in Table 6. The decrease in lipase effect could be attributed to the effect of the mineral ions in the salt enzyme synthesis. Heavy positive ions interact with SH groups and imidazole and carboxyl groups at the active site of the enzyme, resulting in loss of enzyme activity.[26]

The activity of protease increased when treated with Ca^{2+} and Zn^{2+}, which indicates that it plays a role in the stimulation and stability of the enzyme. These results show that the activity of zinc, calcium, and sodium ions in increasing enzymatic efficiency. Many studies have shown that magnesium ion, when added to the medium, increases the stability of the enzyme and also retains the enzyme effectively when zinc and calcium ions exist as shown in Table 6 and 7. The enzyme is more effective in the presence of certain types of ions that are the basis of the enzyme's effectiveness and interferes with the synthesis of the enzyme, especially sodium ions, calcium, zinc, and magnesium, which

Table 5: Extraction and purification of lipase enzyme

Step	Volume (ml)	Activity (unit/ml)	Total activity (unit)	Protein concentration(mg/ml)	Specific activity	Purification on fold	Yield %
The extracted enzyme	90	4.4	4669	13	55	1	100
Deposition with aluminum sulfate	30	12	1850	5	66	2.9	40
Ion-exchange chromatography DEAE-cellulose	13	35	1680	2	52	7.9	36

DEAE: Diethylaminoethyl

Table 6: The effect of some metallic ions and reduce agents in the activity of protease enzyme

Material	Concentration (mM)	Activity %
Control	-	100
$CaCl_2$	10	130
$HgCl_2$	10	32
$ZnSO_4$	10	25
KCl	10	10
$MgSO_4$	10	85
EDTA	5	30
NaN_3	10	19
SDS	5	22
Cysteine	5	99

Table 7: The effect of some metallic ions and reduce agents in the activity of lipase enzyme

Material	Concentration (mM)	Activity %
Control	-	100
$CaCl_2$	10	120
$HgCl_2$	10	36
$ZnSO_4$	10	45
KCl	10	10
$MgSO_4$	10	95
EDTA	5	30
NaN_3	10	19
SDS	5	27
Cysteine	5	99

activate the enzyme and raise the values of enzymatic effectiveness.[27]

CONCLUSION

The enzyme Activity was decremented in these optimum conditions and The activity of the enzyme has increased when treated with Ca ions, which shows that Ca^{++} plays a role in the stimulation and stability of the enzyme, and did not inhibit the activity of the enzyme with the presence of reduced agents such as cysteine, while decrease the enzyme's effectiveness of the factors EDT.

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