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Article

Antibacterial and anticancer activities of (free and immobilized) elastase produced by *Klebsiella pneumoniae*

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Abstract: Elastase is a type of protease that degrades explicitly elastin. The elastase produced by *Klebsiella pneumoniae* isolates was purified by three steps: ammonium sulfate precipitation, ion-exchange chromatography, and Sephadex G-150 chromatography. The optimal condition for elastase production showed high specific activity with starch (3.8 U/mg protein) and casein as a nitrogen source with a specific activity reaching (3.3 U/mg protein). The maximum elastase production was obtained when the pH value was (7.5) with specific activity (4.4 U/mg protein). Elastase (free and immobilized on TiO_{2}- NPs) was used in application as antibacterial and anticancer, and results showed high antibacterial activity against pathogenic isolates, especially *Lactobacillus acidophilus* and *Pseudomonas aeruginosa* were affected by immobilized elastase. Free and immobilized elastase have anticancer activity against lung cancer using the A549 cell line, and immobilized elastase had the potent cytotoxic effect on A549 cells with IC50 142.8 µg/ ml compared with IC50 of normal cell line HdFn on 655.0 µg /ml.

Key Words: *Klebsiella pneumoniae,* Elastase, Immobilization, TiO2-Nps- Antibacterial, Anticancer.

Introduction

Klebsiella pneumoniae is a Gram-negative that belongs to the Enterobacteriaceae family. It usually occurs in healthy humans, including gastrointestinal tracts [1].

Elastase is an enzyme from the protease class that breaks down and degrades elastin. An elastic fiber that, together with collagen, determines the mechanical features of connective tissue [2]. Elastase is one of the proteolytic enzymes (also termed peptidases, proteases and proteinases) capable of hydrolyzing peptide bonds in proteins. Elastases belong to the families of aspartic, cysteine, serine, and metalloproteases. There are many distinct forms of elastases in mammals. [3]. Some microbes, such as Clostridium, Vibrio, Pseudomonas, Aeromonas, Bacillus, Streptomyces, and Aspergillus, have elastases with proteolytic activity [4].

Novel antibacterial drugs should be added to the arsenal to combat multidrug-resistant (MDR) microorganisms, especially before they evolve new resistance

mechanisms against already sensitive antibiotics, in order to save public health [5]. Proteolytic enzymes have significant medical and pharmaceutical importance due to their key role in biological processes and the life cycle of many pathogens [6]. Because of their improved antimicrobial action and extended longevity, nanoscale antibacterial materials have received much attention in recent years as additional or alternative agents [7]. This study aimed to evaluate the effect of elastase enzyme (free and immobilized) - purified from Klebsiella pneumoniae isolates- as an antibacterial against pathogens and an anticancer in vitro.

Materials and Methods

Isolation and identification of K. pneumoniae isolates

Clinical samples (163) were collected from the 15th of April to the 10th of July 2021 from Hospitals in the center of Wasit Governorate- Iraq. These samples included: (burns and wounds, Urine, and sputum). Isolation and identification of K. pneumoniae isolates were cultured on McConkey agar plates in a septic procedure and incubated at 37°C for 24 hours [8]. Then, Gram stain was used to study morphological features (Atlas et al., 1995). After that, 16S rRNA analysis was used to diagnose the target genes [9].

Screening of elastase production by Quantitative Assay

Forty-eight K. pneumoniae isolates were cultured in brain heart infusion broth medium at 37 °C for 24 hours in a shaking incubator *(* at 200 rpm). Cells were removed by centrifugation at 10000 rpm for 10 min and then sterilized with a Millipore filter (0.22µm); 1 ml sterile supernatant was incubated with 20 µg elastin Congo red (ECR) for 30 minutes in a shaking incubator *(* at 200 rpm*).* Then, insoluble ECR was removed by centrifugation at 10000 rpm for 10 min at room temperature, and the absorbance was measured at 495 nm. Fresh BHI medium was used as the blank control [10].

Optimization of Elastase Production

Optimization of parameters for elastase production by using the composition of the minimal medium and 1% elastin substrate**,** then added nitrogen sources (casein, peptone, ammonium sulfate, yeast extract and meat extract), Various carbon sources (glucose, maltose, sucrose, glycerol and starch**),** at a concentration of 1% for the production of elastase, the best nitrogen and carbon sources were adjusted with different pH values (6, 6.5, 7, 7.5, 8, 8.5) and incubated at different temperature (25, 30, 35, 37, 40)°C**.** The culture medium was inoculated with 1% isolate and incubated at 37°C for different periods (24, 48, 72) hrs, and this culture was used to measure elastase production [11].

Extraction and Purification of Elastase

Crude extracellular elastase was extracted from a maximum producer isolate of K. pneumoniae (NJ22) after growing in optimum conditions (24 hours, centrifugation at (10000 rpm) for 10 minutes), and the supernatant was used. At 4 Cº, solid ammonium sulfate was gradually added to 75 ml of crude enzyme at saturation ratios (20- 90 percent), and the enzyme activity content was measured [12].

Purification by Ion exchange chromatography

DEAE-cellulose column (2 x20 cm) was used to purify elastase, and the flow rate was 30ml/hr throughout the column, and the absorbance of each fraction was measured using a spectrophotometer at 280 nm [13]

Purification by Gel filtration chromatography

The Sephadex G-150 (3 x 25 cm) column was used. Phosphate buffer pH8 was used for equilibration and elution at a flow rate of 30 ml/hr, and the absorbance of each fraction was measured using a spectrophotometer at 280 nm.

Elastase activity assay

One ml sterile supernatant was incubated with 1 ml of elastin Congo red buffer (20mg ECR in 6 ml Tris- HCL buffer of 200 mM, pH 8) for 1 h in a water bath at 37°C. To stop the reaction, 0.1M of NaOH was added. Then, insoluble ECR was removed by centrifugation at 10000 rpm for 10 min at room temperature, and the absorbance was measured at 495 nm. Tris-HCL buffer (200 Mm, pH8) was used as a control group. One unit of elastase activity was defined as the amount of enzyme required to hydrolyze 1 mg of elastin per minute at 37C [12].

Elastase immobilization on TiO2 nanoparticles

Adsorption of elastase on TiO2 nanoparticles was done by incubating 1 ml enzyme (containing 86 U/mg, dissolved in 50 mM sodium acetate buffer, pH 4.8) with 10 mg TiO2 nanoparticles suspended in 1 ml of the buffer, as mentioned earlier. The mixture was incubated at 25°C for 3 hours with constant shaking after being centrifuged at 2000 rpm for 5 minutes at 4°C. The adsorbed enzyme-coated TiO2 nanoparticles were washed in a 50 mM sodium acetate buffer (pH 4.8) containing 1 M NaCl and 50% ethylene glycol. The enzyme activity was determined in the supernatant and washings [14]. Characterization of immobilized elastase was determined by using UV-Vis spectroscopy and FTIR analysis.

Antibacterial activity of free and immobilized elastase on TiO2NPS

The agar well diffusion method was used to detect the antibacterial activity of free and immobilized elastase produced by Klebsiella pneumoniae (NJ22) as mentioned in [15] Pathogenic bacteria used in this assay was gram-negative (Klebsiella pneumoniae, E.coli and pseudomonas aeruginosa), and gram-positive, (Streptococcus pneumonia and lactobacillus acidophilus).

Anticancer effect of free and immobilized elastase with TiO2- NPS

MTT assay was done for cytotoxicity analysis on the normal (HdFn) and lung cancer cell lines (A549). Medium of RPMI 1640 was used with different concentrations of free and immobilized elastase (12.5,25,50,100, 200 and 400, μg/mL). Cell viability could be evaluated as follows:

Total Cell Count mL^{-1} = Cell count x Dilution Factor (Sample Volume) x 10^4

Results

Characterization of K pneumoniae

Initial identification of 48 isolates (29.44 %) of K. pneumoniae based on morphological characteristics on MacConkey agar, which appeared mucoid, large, and pink on MacConkey agar due to lactose fermenting. The microscopic

examination of K. pneumoniae isolates is gram-negative. The 16S rRNA gene was revealed in all isolates of K. pneumoniae (100%), as in Figure 1.

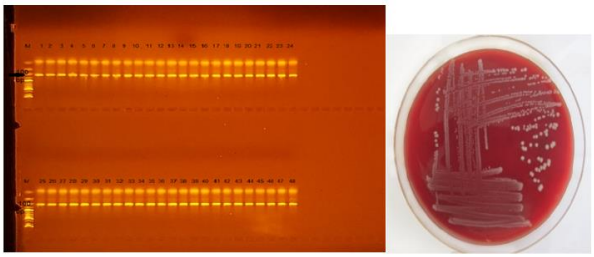

Figure 1. Identification of *Klebsiella pneumoniae. A*- 16SrRNA, *B*-on MacConkey agar medium.

Screening the ability of K. pneumoniae isolates in elastase production

Figure 2 demonstrates that all isolates could generate the enzyme with specific activities ranging from (0.003-1.613) U/mg protein. However, the isolate symbolled (NJ22) has the highest specific activity of 1.613 U/mg protein and is being chosen for future study.

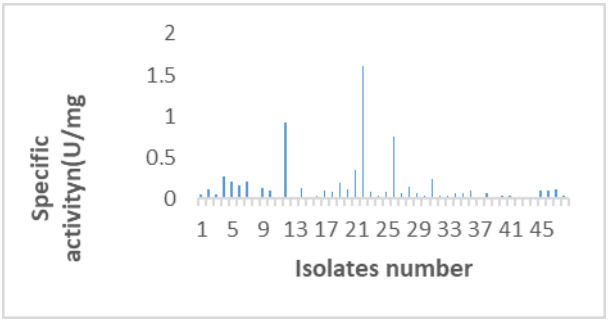

Figure 2. The specific activity of elastase produced by isolates of *K. pneumoniae* after 24hrs incubation at 37°C. On elastin nutrient broth medium.

Optimal nitrogen source for elastase production

Casein was found to be effective in producing the enzyme from K. pneumoniae (NJ22) with a specific activity reaching 3.3 U/mg protein Figure 3. Peptone was the second nitrogen source suitable for elastase production, which gave a specific activity of 2.7 U/mg protein. Yeast extract represented the low nitrogen source for enzyme production of 0.55 U/mg protein.

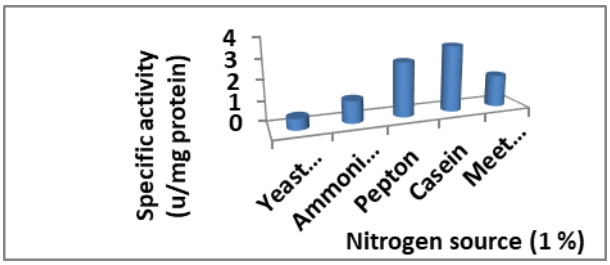

Figure 3. Optimal nitrogen source for elastase production by *K. pneumoniae* (NJ22) incubated at 37°C for 24 hrs.

Because nitrogen provides the microorganism with the building blocks of organic molecules such as proteins, the availability of a nitrogen supply is essential for enzyme production [16].

Optimal carbon source for elastase production

Elastase enzyme showed high specific activity with starch of 3.8 U/mg protein, then decreased with other carbon sources until it reached the low specific activity of 0.8 U/mg protein with glucose Figure 4. Therefore, starch was used as the primary carbon source in later experiments.

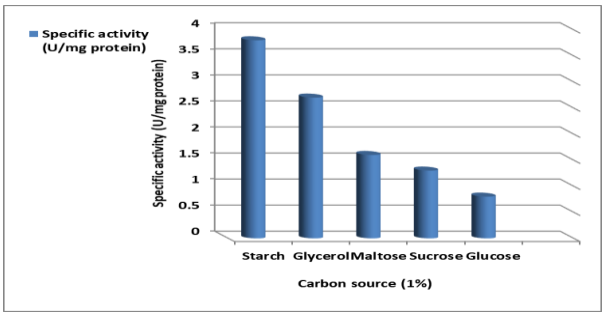

Figure 4. The optimum carbon source for elastase production by *K. pneumoniae* (NJ22).

Optimal pH of medium elastase production

The maximum elastase production was obtained when the pH value of the production medium was adjusted to 7.5. At this value, the enzyme-specific activity recorded 4.4 U/mg protein (Figure 5).

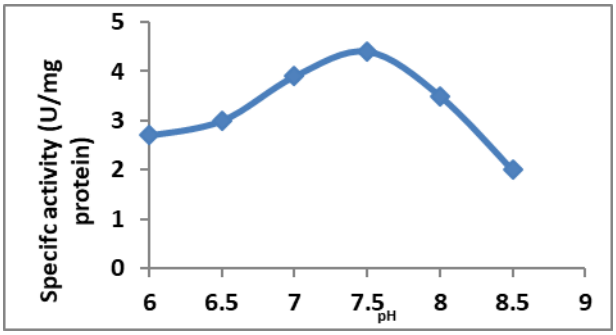

Figure 5. Optimum pH for elastase production by K. pneumoniae (NJ22).

Optimal incubation temperature for elastase production

The specific activity was 3.8 U/mg protein at 37º C. At this temperature, maximum elastase production was observed (Fig. 6). This result, similar to the result recorded by [20], exhibited that the highest elastase production from P. aeruginosa was at 37°C temperature.

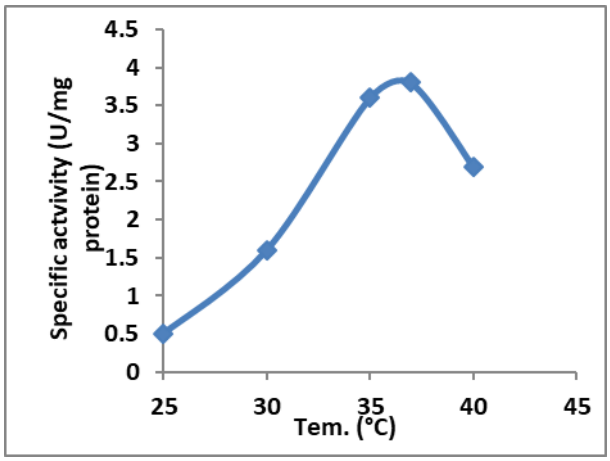

Figure 6. Effect of incubation temperature on elastase production by *K. pneumoniae.*

Optimal incubation period for elastase production

Results in Figure 7 indicated that the specific activity of enzyme production reached the maximum at 4.5 U/mg protein at 24 hr.

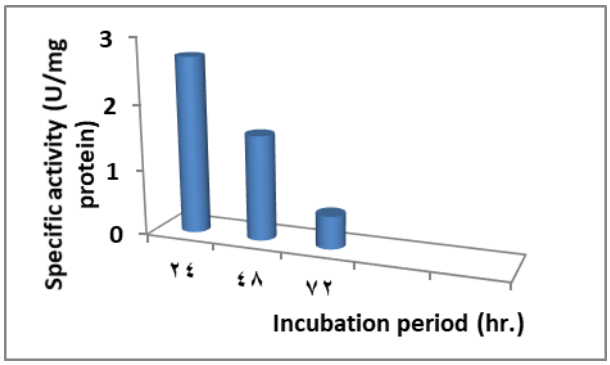

Figure 7. Effect of incubation period on elastase production by *K. pneumoniae* (NJ22).

Purification of elastase

The best enzyme precipitation when ammonium sulfate saturation was (60%) when applied with the crude enzyme (supernatant). The result of the DEAE-cellulose ion exchange chromatography column shown in Figure 8 shows one protein peak in the wash step, and another protein peak appeared after elution in (Fractions 47 to 53) which contained most of the elastase activity, and the enzyme-specific activity was measured to be 36 U\mg protein.

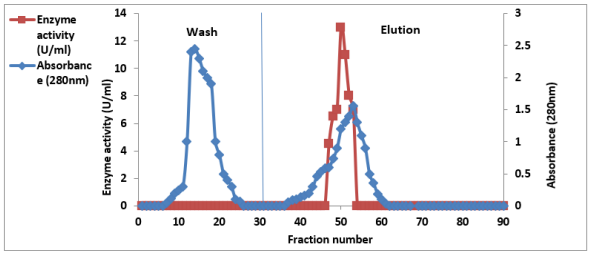

Figure 8. Ion exchange chromatography of elastase from *K. pneumoniae* (NJ22).

After using Sephadex G-150 for further purification of elastase, the results in Figure 9 showed that the high specific activity of the purified enzyme reached 96 U/mg protein.

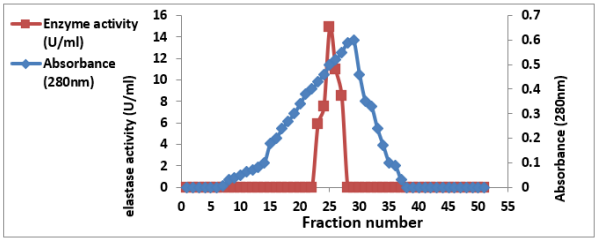

Figure 9. Gel filtration chromatography of elastase from *K. pneumoniae* (NJ22).

12 recorded the purification of elastase from Chryseobacterium indologenes using different procedures of ammonium sulfate and gel filtration on the Sephadex G-75 column, which gave a final purification fold of 8.3 and 5.8% yield. The specific activity was 170 U/mg with purification folds of 39.2 U/mg and(8.8%) overall yield time of elastase purified from P. aeruginosa recorded by [26].

Characterization of immobilized elastase

Figure (10, A) showed UV-visible spectroscopy analysis for immobilized elastase compared with free enzyme and nanoparticles. An absorption peak at 362 nm appeared, which indicates the presence of TiO2 NPs, and an absorption peak of the enzyme at 281nm (Figure 10, B). At the same time, two absorbance peaks were exhibited for elastase immobilized on TiO2-NPs at 280 and 360 nm (Figure C).

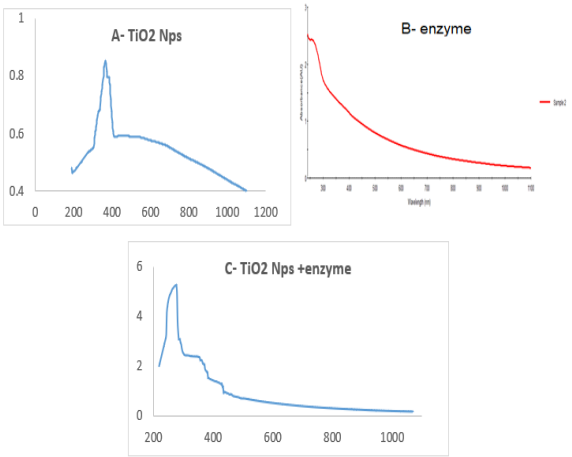

Figure 10: UV−visible absorption spectrum (A)TiO2NPs (B)elastase (C) enzyme +TiO2 NPs.

(Figure 11 a, b and c) FTIR spectra of TiO2 showed that TiO2 Nanoparticles peak in 575 cm^{-1}, characteristic of the O-Ti-O bond. The broadband is observed at 1171.15 cm^{-1}, corresponding to the C=O stretch region shown in Figure (11, a). While the peak for elastase at 3435 cm^{-1} reflected an OH functional group along with an H-bounded vibration. In addition, a detected peak at 2930.40 cm^{-1} belongs to NH primary and secondary amines and amides with higher strength.

The peak at 2327.79 and 2359.32 cm^{-1} corresponded to carbonyl groups, and the peak at 599.62 for NH_2 bonds is shown in Figure (11, b).

Spectra of TiO_2NPs with immobilized elastase showed familiar characteristic peaks, while several differences existed due to slight chemical interaction of elastase within the TiO_2NPs conjugate. As shown in Figure (11 c), new peaks were generated and appeared in FTIR spectra of the immobilized enzyme, which were not TiO2NPs spectra; the strong NH group with a peak at 3435.06 cm^{-1} indicated the presence of elastase on the TiO2NPs, the carbonyl group at 2955.23 and 2881.65 cm^{-1}. These new peaks in the elastase conjugate TiO2NPs confirmed the successful synthesis of the enzyme immobilized with nanoparticles.

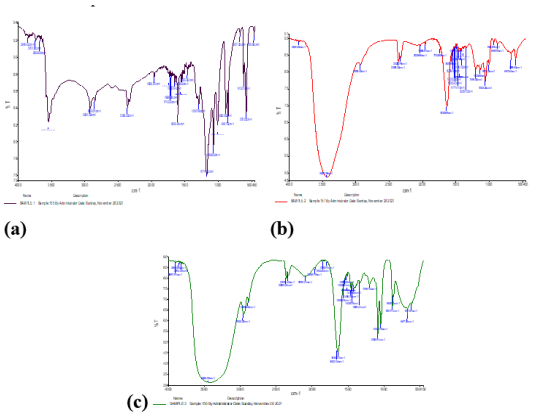

Figure 11. FT-IR analysis of (a) TiO2NPs, (b) elastase and (c) immobilized elastase.

Antibacterial activity of purified free and immobilized elastase

The results indicated that free and immobilized enzymes possess significant antibacterial activity against all pathogenic isolates in contrast with control. The antibacterial activity of immobilized elastase was significantly higher than free enzyme and TiO2 NPs against all isolates, as shown in Figure (12). Lactobacillus acidophilus and Pseudomonas aeruginosa were the most bacterial isolates affected by immobilized elastase, followed by E. coli and Klebsiella pneumoniae, with inhibition zones of 26, 11 and 9 mm, respectively, at the highest concentration.

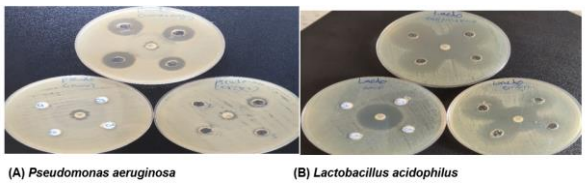

Figure 12. Antibacterial activity of free and immobilized elastase against pathogenic bacteria.

Anticancer activity of purified free and immobilized elastase

The anticancer effect of free elastase was evaluated against the A549 cell line as well as the normal HdFn cells using MTT assay for 24 hrs., with a reduction rate of 69.98 ± 2.18 to 94.79± 2.81 from the concentrations 400 to 12.50 µg ml. It showed that the elastase enzyme exhibited significantly the most potent cytotoxic activity with an IC50 value of 243.8 µg/ml compared to IC50 of 384.5 µg/ml on the HdFn regular cell line Figure 13.

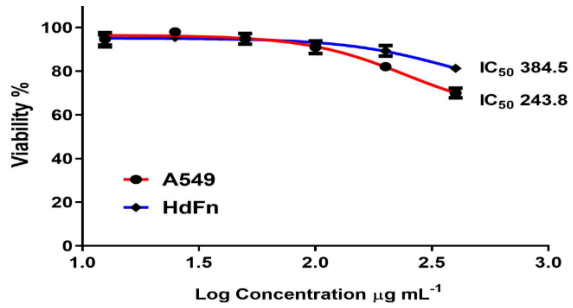

Figure 13. Cytotoxic effect of elastase on A549 and HdFn cells.

The cell viability is reduced by increasing the concentration of immobilized elastase enzyme. The decrease in A549 cell viability was noted by 400µg/ml (55.71 ± 3.60), while the highest A549 cell viability at 12.50 µg/ml reached (94.87 ± 0.29). Figure 14 showed that immobilized elastase had a potent cytotoxic effect on A549 cells, and the IC50 value of immobilized enzyme was 142.8 µg/ ml, while IC50 of regular cell line HdFn was 655.0 µg /ml.

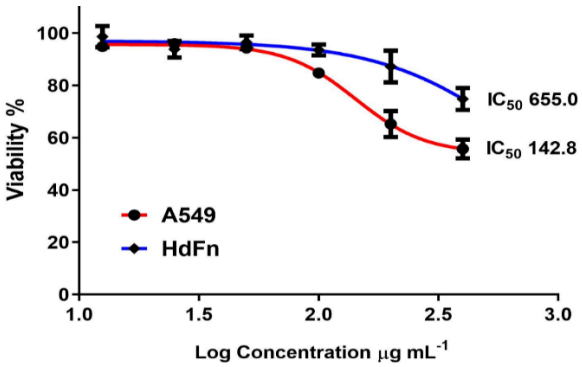

Figure 14. Cytotoxic effect of immobilized elastase on A549 and HdFn cells after 24 hrs incubation at 37 ºC.

Cancer is a mutation-driven disease with significant geographical and temporal genetic variability (Vogelstein et al., 2013). Finding medicines that combine broad efficacy across cancer types with selectivity to limit host damage has been difficult. The fundamental idea behind enzyme therapy methods is to use enzymes capable of depleting cells of a specific type of amino acid required for malignant cell growth but not for average cell survival.

Discussion

The cell-free extracts of K. pneumoniae isolates were tested spectrophotometrically using a method reported earlier by [10] to detect the effective bacterial isolates in elastase production.

P. aeruginosa requires that to induce elastase and enable growth on casein when casein is the sole nitrogen and carbon source [17]. The use of several carbon sources by Klebsiella sp. to synthesize an extracellular biosurfactant was investigated. The largest generation of crude biosurfactant was discovered with starch among the several carbon substrates examined [18]. The elastase enzyme production by microorganisms has been reported to be enhanced by carbon

source and other operating parameters [19]. These results agree with [20], in which most elastase from P. aeruginosae exhibited optimum pH values ranging from 7 to 9. The pH of culture media changes when the hydrogen ions (H+) concentration lowers or rises. Because H+ and OH compete with hydrogen and ionic bonds in an enzyme, this can result in the denaturation of the enzyme [21]. On the other hand, the ionization states of the substrate or amino acid side chain are altered by pH, which is a determining factor in the expression of enzyme activity.

However, [23] showed that the best temperature for the production of Elastase from Bacillus sp. was 30°C. This variance may be due to the difference in bacterial isolate or to geographical factors.

This result is consistent with [13], who found that P. aeruginosa produces the most elastase after 24 hours of shaking incubation. The decrease in elastase production after 24 hours could be attributed to a lack of nutrients in the medium, the accumulation of excess acid in the media due to sugar utilization, or the development of oxygen tension. It was also related to the development of acetate, which inhibits cell growth and causes the production of toxic chemicals, resulting in lower protein production [24].

The results showed that the protein had 76.6 % overall yield and 8.2 purification fold after purification by DEAE cellulose. Ion chromatography separates ions and polar compounds based on their affinity for the ion exchanger. It operates on large proteins, tiny nucleotides, and amino acids, among other charged molecules. However, ion chromatography must be done in conditions that are one unit away from the isoelectric point of a protein [25].

Nanoparticles have also been shown to detect bacteria that cause infections in samples quickly and with greater accuracy than traditional methods [27]. The major types of antimicrobial enzymes are proteolytic enzymes and polysaccharide-degrading enzymes among the proteolysis enzymes, which belong to the serine protease [28]. Moreover, a single amino acid deficiency has been identified as a potentially effective cancer treatment method [29].

Conclusion

The results of the present investigation revealed that the Klebsiella pneumoniae bacteria are a potential source of elastase enzymes. High specific activity for elastase purified from Klebsiella pneumoniae (NJ22) is obtained after three purification steps by ammonium sulfate precipitation (60%), DEAE-Cellulose, and Sephadex G150. It is concluded from the present study that the elastase enzyme isolated from bacterial strains possesses significant antibacterial activity against gram-negative and gram-positive bacteria. Free and immobilized elastase had cytotoxic activity against the A549 cancer cell line.

Acknowledgment

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References

1 Martin RM, Bachman MA. Colonization, infection, and the accessory genome of Klebsiella pneumoniae. *Frontiers in Cellular and Infection Microbiology*. **2018***;8(JAN):*1-15. doi:10.3389/fcimb.2018.00004

2 Everett MJ, Davies DT. Pseudomonas aeruginosa elastase (LasB) as a therapeutic target. *Drug Discovery Today*. Published online **2021**.

3 Fujii T, Fukano K, Hirano K, et al. A new serine protease family with elastase activity is produced by Streptomyces bacteria. *Microbiology (Reading, England)*. **2020**;*166(3):*253-261. doi:10.1099/mic.0.000880

4 He GQ, Chen QH, Zhang L, Liu XJ. Influence of medium components on elastase production using crude sources by Bacillus sp. EL31410. *Journal of Zhejiang University Science*. **2003**;*4(2):*142-151. doi:10.1631/jzus.2003.0142

5 Culp EJ, Waglechner N, Wang W, et al. Evolution-guided discovery of antibiotics that inhibit peptidoglycan remodeling. *Nature*. **2020**;*578(7796):*582-587.

6 Mótyán J, Tóth F, Tőzsér J. Research Applications of Proteolytic Enzymes in Molecular Biology. *Biomolecules*. **2013**;*3(4):*923-942. doi:10.3390/biom3040923

7 Loh KP, Bao Q, Ang PK, Yang J. The chemistry of graphene. *Journal of Materials Chemistry*. **2010**;*20(12):*2277-2289.

8 Patel SS, Chauhan HC, Patel AC, et al. Isolation and Identification of Klebsiella pneumoniae from Sheep-Case Report. *International Journal of Current Microbiology and Applied Sciences*. **2017**;*6(5):*331-334. doi:10.20546/ijcmas.2017.605.037

9 Abbas ON, Mhawesh AA, Al-Shaibani AB. Molecular identification of pathogenic klebsiella pneumoniae strains producing biofilm. *Medico-Legal Update*. **2020**;*20(3):*1068-1074. doi:10.37506/mlu.v20i3.1544

10 Li J, Ramezanpour M, Fong SA, et al. Pseudomonas aeruginosa Exoprotein-Induced Barrier Disruption Correlates With Elastase Activity and Marks Chronic Rhinosinusitis Severity. *Frontiers in Cellular and Infection Microbiology*. **2019**;*9(February):*1-9. doi:10.3389/fcimb.2019.00038

11 Kumar D, Kumar A, George N, Gupta N. Production optimization and characterization of an alkaline thermostable protease and its application as a laundry additive. **2016**;(6):9-43.

12 Lei Y, Zhao P, Li C, Zhao H, Shan Z, Wu Q. Isolation, identification and characterization of a novel elastase from Chryseobacterium indologenes. *Applied Biological Chemistry*. **2018**;*61(3):*365-372. doi:10.1007/s13765-018-0364-6

13 Shinji T, Moe Y, Yukihiro K, Yoko Y, Hitoshi A. Characterization of an organic-solvent-stable elastase from Pseudomonas indica and its potential use in eggshell membrane hydrolysis. *Process Biochemistry*. **2019**;*85(June):*156-163. doi:10.1016/j.procbio.2019.06.021

14 Ahmad R, Sardar M. Immobilization of cellulase on TiO2 nanoparticles by physical and covalent methods: A comparative study. *Indian Journal of Biochemistry and Biophysics*. **2014**;*51(4)*:314-320.

15 Indarmawan T, Mustopa AZ, Budiarto BR, Tarman K. Antibacterial Activity of Extracellular Protease Isolated From an Algicolous Fungus Xylaria psidii KT30 Against Gram-Positive Bacteria. *HAYATI Journal of Biosciences*. **2016**;*23(2)*:73-78. doi:10.1016/j.hjb.2016.06.005

16 Pant G, Prakash A, Pavani JVP, et al. Production, optimization and partial purification of protease from Bacillus subtilis . *Journal of Taibah University for Science*. **2015**;*9(1):*50-55. doi:10.1016/j.jtusci.2014.04.010

17 Yan H, Wang M, Sun F, Dandekar AA, Shen D, Li N. A metabolic trade-off modulates the policing of social cheaters in populations of Pseudomonas aeruginosa. *Frontiers in Microbiology*. **2018**;*9(FEB):*1-10. doi:10.3389/fmicb.2018.00337

18 Jain RM, Mody K, Joshi N, Mishra A, Jha B. Production and structural characterization of biosurfactant produced by an alkaliphilic bacterium, Klebsiella sp.: Evaluation of different carbon sources. *Colloids and Surfaces B: Biointerfaces*. **2013**;*108*:199-204. doi:10.1016/j.colsurfb.2013.03.002

19 He GQ, Chen QH, Zhang L, Liu XJ. Influence of medium components on elastase production using crude sources by Bacillus sp. EL31410. *Journal of Zhejiang University: Science*. **2003**;*4(2):*142-151. doi:10.1631/jzus.2003.0142

20 Ghorbel-Bellaaj O, Hayet BK, Bayoudh A, et al. Pseudomonas aeruginosa A2 elastase: Purification, characterization and biotechnological applications. *International Journal of Biological Macromolecules*. **2012**;*50(3):*679-686. doi:10.1016/j.ijbiomac.2012.01.038

21 Bhunia B, Basak B, Mandal T, Bhattacharya P, Dey A. Effect of pH and temperature on stability and kinetics of novel extracellular serine alkaline protease (70kDa). *International Journal of Biological Macromolecules*. **2013**;*54(1):*1-8. doi:10.1016/j.ijbiomac.2012.11.024

22 Turner BL, Romero TE. Stability of hydrolytic enzyme activity and microbial phosphorus during storage of tropical rain forest soils. *Soil Biology and Biochemistry*. **2010**;*42(3):*459-465. doi:10.1016/j.soilbio.2009.11.029

23 He GQ, Xu Y, Chen QH, Ruan H, Li JJ. Effect of temperature on batch elastase production by Bacillus sp. EL31410. *Journal of Zhejiang University: Science*. **2004**;*5(12):*1583-1589. doi:10.1631/jzus.2004.1583

24 El-Naggar NEA, El-Ewasy SM. Bioproduction, characterization, anticancer and antioxidant activities of extracellular melanin pigment produced by newly isolated microbial cell factories Streptomyces glaucescens NEAE-H. *Scientific Reports*. **2017**;*7(September 2016)*:1-19. doi:10.1038/srep42129

25 Din NAS, Lim SJ, Maskat MY, Abd Mutalib S, Zaini NAM. Lactic acid separation and recovery from fermentation broth by ion-exchange resin: A review. *Bioresources and Bioprocessing*. **2021**;*8(1):*1-23.

26 Kotb E, El-Zawahry YA, Saleh GE. **Isolation** of a putative virulence agent, cytotoxic serine-elastase, from a newly isolated Pseudomonas aeruginosa ZuhP13. *Journal of Biosciences*. 2019;*44(1).* doi:10.1007/s12038-018-9829-3

27 Sundaramoorthy NS, Nagarajan S. Can Nanoparticles Help in the Battle against Drug-Resistant Bacterial Infections in "Post-Antibiotic Era"? In: *Antimicrobial Resistance*. Springer; **2022**:175-213.

28 Cao Y, Naseri M, He Y, Xu C, Walsh LJ, Ziora ZM. Non-antibiotic antimicrobial agents to combat biofilm-forming bacteria. *Journal of Global Antimicrobial Resistance*. **2020**;*21*:445-451. doi:10.1016/j.jgar.2019.11.012

29 Zolfaghar M, Amoozegar MA, Khajeh K, Babavalian H, Tebyanian H. Isolation and screening of extracellular anticancer enzymes from halophilic and halotolerant bacteria from different saline environments in Iran. *Molecular Biology Reports*. **2019**;*46(3):*3275-3286. doi:10.1007/s11033-019-04787-7

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