RESEARCH ARTICLE

Cytotoxic Effect of Pseudomonas aurogenosa Protease on Cancer Cells

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ABSTRACT

About (20) *Pseudomonas rogenosa* isolate was experienced for their ability of protease production by calculating the diameter of lysis area after developing on skim milk agar medium (qualitatively). The results exhibited that only isolate no 5 was higher isolate for protease making of (26mm) of lysis area.

Then, the protein concentration was also identified by the Bradford method and found of 0.16 mg/mL; then purification was done by using an ion-exchange chromatography with DEAE sephadex G-100 column. The results showed the presence of 1-peak of the enzyme with 50 Kd of molecular weight two peaks of other proteins. We tried to investigate the cytotoxic invitro effect of the purified enzyme against two human cancer lines, HeP2 (human larynx epidermed carcinoma), RD (Rabdo- Sarcoma), and one normal cell line Ref (Rat embryonic fibroblast) . The cancer and normal cells were treated with different concentrations of protease enzyme ranging from (0.05, 0.1, 0.2, 0.4,0.8 and 0.16 mg/mL) then incubated for additional 48 hours at 37^0C and the results showed highest toxicity (80.28%) of protease enzyme on RD , moderate cytotoxicity (45.52%) on Hep and slight toxicity (37.12%) on normal cell line (Ref) in a concentration (0.8mg/mL).

Keywords: Protease, *Pseudomonas rogenosa,* Cancer cells.

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INTRODUCTION

Pseudomonas aeroginosa is the most Common gram-negative organism causing nosocomial infections and is a noteworthy pathogen in immune and myelosuppressed patients. It is non-fermentative and typically aerobic however will develop under an aerobic conditions in the presence of an appropriate nitrogen source. So, it very well may be isolated from soil, water, wounds, dental tissues.[1] The living being likewise can expound an expansive number of secretory items and proteins, including extracellular protease.[2,3] Characterize essential virulence factors and and have the capability to effect on IgA and IgG immunoglobulins.[4]

Two main potent proteases have been isolated, portrayed and widely studied *Pseudomonas* elates (PE) and alkaline protease (PAP). *Pseudomonas*, isolated from an assortment of ecological sources and tissues infected patients secret these protease,[5] which are probably instrumental in imitating and controlling the tissue invasion and necrosis characteristic of *pseudomonas* infections.[6]

Separate of cancer cells industriously affects strong tumors or submerge the blood with abnormal cells. Cell division is a typical method used via the body for repair and growth.[7] Parent cells divided to make two daughter cells. And these daughter cells are used to form novel tissue, or to altercation cells that have died consequently of aging or harm. Stop splitting of healthy cells when there is no lengthy required for more daughter cells, but cells of cancer remain to yield copies. They are also able to spread starting with one a player in the body then onto the next in a procedure known as metastasis.[8]

MATERIAL AND METHODS

P.aerogenosa isolates: they were isolated from wound infections.

Bacterial isolation

Every one of the synthetic compounds has gotten from sigma concoction Co. (USA) and (BDH England). The cotton swap of wound examples was spread on blood agar and McConkey agar plates, brood at 37°C medium-term. At that point, single states which are non-lactose aged and gives G +ve response on oxidase test and catalase test, were exchanged to Macconkey agar and blood agar to get an unadulterated culture, at that point various biochemical tests and even VITEK2 minimized framework were done.[9]

Protease production assay

Qualitative method

CLED or Nutrient agar plates having skim milk (5–10%) inoculated with bacterial overnight developing a culture at 37°C. The lysis cleared area around the growing colonies

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represent the positive result of protease production.[10] Then the Azocasimase assay, were used to define the enzyme activity and,[11] method for determination of specific activity and protein concentration.

Preparation of partially purified protease

Incompletely partially protease were set up as indicated by[12] by inoculation of sterile dialysis tubing on the surface of blood agar by a swab with medium-term societies (37°C) of Proteus vulgaris 16 from nutrient broth. The plates were brooded medium-term at 37°C. The bacteria were scratched from the films with a glass slide and suspended in 50Mm (tris-HCL (pH 8.0) containing 0.04% NaN_3. The layers were then completely washed in this cradle, and the washings were added to the bacterial suspension, centrifugation, the supernatant containing extracellular protease was added to 60% (wt/vol) Ammonium sulfate mixed at 4°C medium-term, the hastened proteins were gathered by centrifugation and redissolved in tris–HCL and 100 M NaCl (pH8.0) and connected to the section DEAE with 100Mm Nacl.

CELL LINE GROWTH AND CYTOTOXICITY ASSAY

Cell culture: Hep2 (Human Larynx Epidermoid carcinoma), RD (Rhabdo-myasarcoma) and Ref (Rat fetus fibroblast) cell lines were acquired from Iraqi community for malignancy research and restorative hereditary, Mustansiriyah University. The cells were developed in RPMI–1640 medium containing 10% fetal calf serum and brooded at 37C0 to blended monolayer.

The connected cells washed firstly with PBS and gathered from the tissue culture flask by treatment with trypsin versine solution. The cells were counted by trypan-blue (about 95% viability), 200µ of cell suspension were harvested in each well of microtiter plate and incubated in CO_2 incubator for 24h at 37°C then 200µL of altered concentrations of purified protease represented in (0.16, 0.8, 0.4, and 0.2, 0.1, 0.05) µg/mL were set up in serum free media (SFM) and added to cells, then re-incubate the plates for additional 48 hours. Moreover, each concentration were duplicated in three wells and negative control wells were treated with SFM just. Toward the finish of introduction period, the cells were recolored by 100µl of crystal violet solution and incubated at 37°C for 30 minutes.[13] The optical density of each well was read by micro–ELISA reader at 492 nm. The percentage of inhibition proportion was calculated agreeing to.[14]

Statistical analysis: The experiments data were analyzed using statistical software SPSS version 16, significant differences between treatments were assessed using students T- test and p values <0.05 were considered significant.

RESULTS & DISCUSSION

From the acquired outcomes, extraction of protease from bacterial cells, it could be presumed that the isolate no 5 demonstrated the higher capacity for protease generation of (26mm) in width of lysis zone on skim drain agar medium with higher protein concentration of 0.16 mg/ml by Bradford technique.as in Table 1.

$$\text{Inhibition rate (R)} = \frac{\text{optical density of control wells} - \text{optical density of test wells} * 100}{\text{optical density of control wells}}$$

Purification of *pseudomonas aerogenosa* protease

Ammonium sulfate preparation:

It was seen that *pseudomonas aerogenosa* protease action was the most concentration of ammonium sulfate (20–90%) but it was more in 50, 60, 70 and 80% saturation.

Chromatography Exchange Ion

The results depicted Figure 1 that there was one protein peak showed up in the washing step with 50 kDa molecular weight, while three primary protein peaks appeared in the elution by gradient concentrations of sodium chloride, the washing fraction contained most of the enzyme activity while there was no activity in the eluted fraction with Nacl solution.

Cell line Growth and Cytotoxic Assay

To examine the effects of *pseudomonas aeroginosa* protease enzyme on the growing of two cell lines (Hep and RD) and standard cell line (Ref), the cells cultures exposed to six different concentrations of the purified protease enzyme (0.05, 0.1, 0.2, 0.4, 0.8, and 0.16) µg/mL. The results showed a significant differences ($p < 0.05$) in percentages of inhibition rate (IR) on Hep2 cell line as compared to control, depending on the concentration of the enzyme the IR was 18.75% at a concentration 0.1µg/mL than increases to 52.64% at a concentration 0.4 µg/mL, than it decreased at higher concentrations of the extract Figure 2 The same pattern of inhibition rate was revealed in the growth of RD cell line, the results illustrated that IR was 45.3% at concentration of 0.1 µg/mL and increased significantly ($p < 0.0.5$) to 80.28% at a concentration of 0.4 µg/mL, and the inhibition rate decreases by increasing concentration of the extract (Figure 3) from these

Table 1: Showed the protease production and protein concentration of Pseudomonas aeuroginosa isolates.

Isolate no	*Diameter in mm*	*Protein concentration mg/mL*
1	20	0.10
2	18	0.9
3	18	0.9
4	20	0.10
5	26	0.16
6	22	0.11
7	20	0.9
8	18	0.9
9	18	0.10
10	20	0.11
11	22	0.9
12	18	0.9
13	18	0.8
14	16	0.9
15	18	0.10
16	20	0.9
17	19	0.8

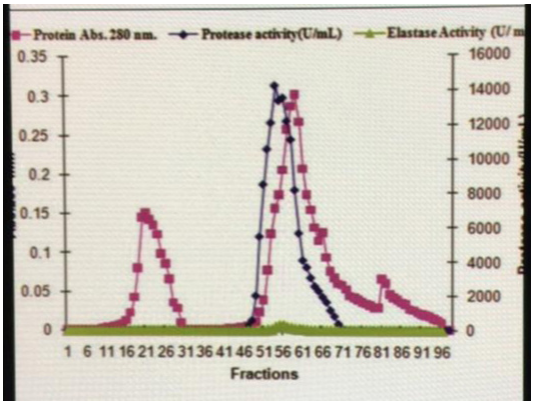

Figure 1: Lon exchange chromatography of protease from p. a using DEAE–Sephadex (2.7 × 7 cm) column fraction volume was 5mL at flow rate of 30ml/hr.

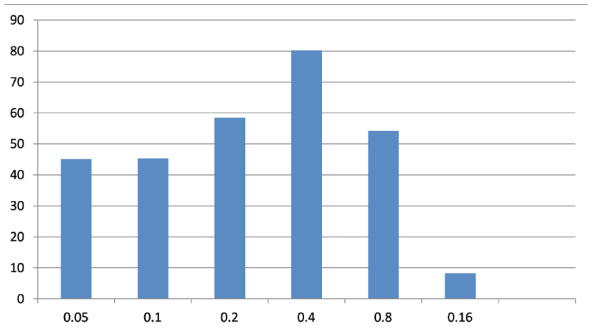

Figure 3: Percentage of cytotoxicity represented by inhibition rate (IR) in RD cell line treated with different concentrations of *Pseudomonas aeruginosa* protease after 48 hours of exposure.

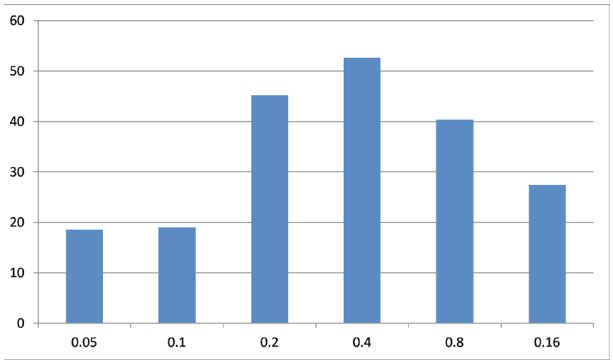

Figure 2: Percentage of cytotxicity represented by inhibition rate (IR) in Hep2 cell line treated with different concentration of *p. a*. protease after 48 hours of exposure.

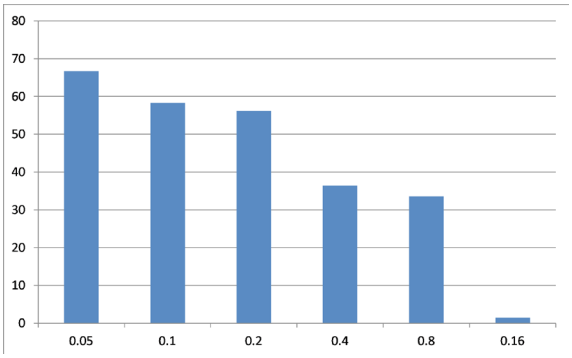

Figure 4: Percentage of cytotoxicity represented by inhibition rate (IR) in REF cell line treated with different concentrations of *p. a*. protease after 48 hours of exposure.

results it is clear that the effects of the active compound obvious in low concentrations. Cultured normal cell line (Ref) showed a significant differences ($p < 0.05$) in percentage of inhibition rate, the highest IR was from 66.6% at low concentration 0.05 µg/mL, and decreased significantly as the concentration of the extract increased, and the lowest IR was 1.64% at concentration 0.16 µg/mL the normal call line (Figure 4). the results were closely related to the results obtained,[15] that is the addition of 50µg/ml of plant increase thymocyte viability by 79.4%-84.3%. It is also proved by that some bacterial enzymes are cytotoxic at a higher concentration toward human normal cells.

From the results obtained it can be concluded that the strongest cytotoxicity of the extract was at a concentration 0.8 µg/ml, since it is effective against Hep and RD cell line and less effective against normal cell line Ref. These results come in agreement with the previously improved results which used the *Rhus spp.* extract as antitumor and antioxidant.[16, 17, 18, 19, 20] The addition of the protease enzyme 5 Ref to the cultured cell line promote cell cycle arrest at either the G0/G1 or G2/M phase rather than causing cell damage damage directly, and eventually induced apoptosis in cell line It was believed that the induction of apoptosis is related to the inhibition of the activity of signal transduction molecules involving in the cell cycle, since certain antioxidants, such as flavonoids, have been reported to exert inhibitory effects on P13-Kinase.[21] As well as may bacterial enzymes have an effect on human cells especially on cancer cells in addition to thein effect on human IgG.[22]

REFRENCES

1 Kaye, K. S., Gales, A. C., & Dubourg, G. (2017). Old antibiotics for multidrug-resistant pathogens: from in vitro activity to clinical outcomes. International journal of antimicrobial agents, 49(5), 542-548.

2 Pavlov, P. I. (2010). Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex. Annals of neurosciences, 17(3), 136.

3 Winstanley C, Brien SO, Brockhurst MA (2016) Pseudomonas aeruginosa evolutionary adaptation and dLversLficatLon in cystic fibrosLs chronic lung infections. Trends Microbiol 24:327-37.

4 Fortier, L. C., & Sekulovic, O. (2013). Importance of prophages to evolution and virulence of bacterial pathogens. Virulence, 4(5), 354-365.

5 Hoge, R., Pelzer, A., Rosenau, F. & Wilhelm, S. (2010). Weapons of a pathogen: proteases and their role in virulence of Pseudomonas aeruginosa. In Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, No. 2, pp. 383–395. Edited by A. Mendez-Vilas. Spain: Formatex Research Center.

6 Trinchieri, G. (2012). Cancer and inflammation: an old intuition with rapidly evolving new concepts. Annual review of immunology, 30, 677-706.

7 Martin, E. A. (Ed.). (2015). Concise medical dictionary. Oxford University Press, USA.

8 Vlassov, A. V., Magdaleno, S., Setterquist, R., & Conrad, R. (2012). Exosomes: current knowledge of their composition,

biological functions, and diagnostic and therapeutic potentials. Biochimica et Biophysica Acta (BBA)-General Subjects, 1820(7), 940-948.

9 Acto, J. K. (2012). Clinical Bacteriology. Elsevier's Integrated Review Immunol.and Microbiol. (2nd edition), Pages 105-120.

10 Loomes, L.M.; Senior, B.W.& Kerr (1990). A Proteolytic enzyme secreted by Proteus mirabilis degrades immunoglobulines of the immunoglobulin A1(IgA1), IgA2& IgG isotypes Infect. Immune.58: 1979-1985.

11 Bradford, M.M. (1976). A rapid and sensitive method for the Quantitation of Microgram Quantities of protein utilizing the principle of protein dye-binding Anal.Biochem.72: 248-254.

12 Higerd, T.B.; Virella, Cardenas, Koistinen, J.W.(1977).New method for obtaining IgA-specific protease. J.Immunol. Methods. 18:245-249.

13 Del Campo. J. Amiot, M J. and Nguyen, C.(2002). Antimicrobial effect of rosemary extract.J. Food Prod;63(10):1359-1368.

14 Delaquis, PJ.; Stanich, K.; Girard, B. and Mazza, G. (2002). Antimicrobial activity of individual and mixed fractions of dill, cllantro, coriander and eucalyptus essential oils, Inter. J Food Microbiol; 74: 100-109.

15 Jeong, C.L., Ju. K. and Yong –Suk, J. (2003).Ethanol eluted extract of Rhus verniciflua stokes inhibits cell growth and induce apoptosis in human lymphoma cells. J. of Biochem and Bio; 36(4): 337-343.

16 Guppata, SN., Pramani, K., S., Tiwar, OP. Thacker, N., Paude, MS., Upmanyu, N. (2010) Immunomodulatory activity of Gymnema sytvestre leaves. The internet J. pharmacol; 8(2): 1.

17 Lee, JC. Lee, KY., Kim, J., Na, CS., Jung, N.C., Chung, GH., Jang, Y. S. (2004) Extract from Rhus verniciflua stokes is capable of inhibiting the growth human lymphoma cells. Food and Chemical Toxicology; 1383–1388.

18 Jeong–Chae, L., Kye–Taek, L. and Yong–Suk, J. (2002). Identification of Rhus verniciflua stocks compounds that exhibit free radical scavenging and anti-apoptotic properties. Biochemical et Biophysica Acta (BBA); 1570(3): 181-191.

19 Kosar, M., Bozan, B., Temelli, F., and Baser, K.H.C., Antioxidant activity and phenolic composition of (Rhus coriaria L.) extracts Food chemistry, 2007; 103 (3):952-959.

20 Lim, K. T., Hu, C., Kitts, D.D., Antioxidant activity of a Rhus verniciflua stokes ethanol extract. Food and Chemical Toxicology, 2001; 39: 229–237.

21 Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. Clinical interventions in aging, 2(2), 219.

22 Joe, Gold Fsky, S.Stephen wood, H: & Sasha, H.S. (2015). Pseudomonas aeruginosa exotoxin induces Potent Cytotoxicity against a variety of murine and human cancer cells.J. Med. Microbiol. 64 (5), 586.