Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 1 Vol 8 No 1 2023

Article

Biological activities of purified prolidase from pathogenic *E.coli*

Seror A.Abdul Hussein*, Neihaya H. Zaki, and Khetam H.Rasool
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.
Correspondence: sroor@esraa.edu.iq , dr.neihayahz@uomustansiriyah.edu.iq , dr.khr@uomustansiriyah.edu.iq
Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.01.57

Abstract: Prolidase is a ubiquitous enzyme that plays a chief role in the metabolism of proline-rich proteins. The goal of this study extraction and purify prolidase from *E. coli* and evaluate some applications such as anti-biofilm and anticancer. Hundred stool Samples were collected from infants with breastfeeding, non-vomiting, and non-diarrhea to isolate *E.coli* bacteria. A 16S rRNA gene (585 bp) was found in all isolates of *E. coli* via PCR identification. Depending on the qualitative method on prolidase agar, only 40 (80%) isolates could produce prolidase from 50 isolates that were considered non-pathogens, then only 32 isolates revealed different levels in prolidase production with specific activity equal to (2.1U/mg) of *E.coli*.
MS12. Sucrose, casein, and 40C^{o} were the chosen isolate's best conditions for producing prolidase. Cold acetone precipitation and dialysis were used to extract the enzyme, and DEAE-cellulose and the Sephadex G-150 column were used in purification with specific activity (2 U/ml) and (6.6 U/mg) protein. Prolidase showed the highest effect on biofilm at 500 µg/ml concentration against *P. aeruginosa*, then *E. coli*, 65% and 60.3% respectively. Brain Tumor Cell Line (A127), Colorectal Adenocarcinoma cells (CaCo-2), and Normal embryonic liver cell line (WRL-68) were used to test the prolidase effect on these cell lines. An assay of MTT was used to detect the inhibiter concentration (IC50) values and cytotoxic effect of purified prolidase.

Keywords: *E.coli*, Prolidase, purification, antibiofilm, anticancer activity.

Introduction

Prolidase is the sole enzyme that can cleave imidodipeptides to proline or hydroxyproline at the C-terminus. Prolidase can be found in archaea, bacteria, and eukaryotes, which means it can be found in all three domains of life [1]. Prolidase has been isolated from archaeal, bacterial, and eucaryotic sources. Prolidase's best-researched substrate is imidodipeptides, formed during collagen degradation, the most abundant extracellular matrix component (ECM). Prolidase is expected to work with other endo- and exopeptidases in bacteria and archaea to break down intracellular proteins and recycle proline [2]. Although the enzyme is known to have protective activity against harmful organophosphates, its physiological purpose in bacteria remains to be determined. [3].

E.coli is a rod-shaped, Gram-negative, facultative anaerobic bacterium from Escherichia. It is typically found in warm-blooded species in the lower intestine [4]. E. coli prolidase has a role similar to human prolidase in the breakdown of dipeptides produced by protein catabolism and a regulatory role. [5]. This study aimed to extract and purify prolidase from non-pathogenic E. coli bacteria and assess its biological applications like antibiofilm and anticancer in vitro.

Materials and methods

Bacterial isolation and identification

Samples of male and female feces from infants (2-7) months, with the attributes of breastfeeding, non-vomiting, and non-diarrhea, were collected from Al-Alwia Children's Hospital between March and September of 2021.

The primary diagnostic of the E. coli by using MacoConkey agar and Eosin methylene blue agar [30]. I then used 16sRNA for a confirmed diagnosis of E.coli by using Sequences (5-3) F- GTGATACAGGTGCTGCTGCATGG and R-TTATGAGATCGCTGCACTCG Using monoplex PCR [29].

Pathogenic microorganisms

Bacteria and fungi (E. coli, Pseudomonas aeuginosa, Staphylococcus epidermidis, Staphylococcus aureus, and candida albican*)* were multi-drug resistance and biofilm formers obtained from a postgraduate laboratory in the Biology department- Mustansiriyah University, cultured on Brain Heart Infusion agar, and incubated at 37 ^{o}C for 24 h.

Detection of prolidase production

The ability of E. coli isolates for prolidase production was examined via using semi-quantitative and quantitative methods: Semi-quantitative analysis on Proline medium was used for all isolated bacteria and incubated at 37 C for 24 hrs., and an appearance of a clear zone around the colonies refer to prolidase production. A quantitative test for bacterial isolate was chosen (as a high inhibition zone), inoculated in proline broth and incubated at 37^{o}C for 24 hours. After centrifugation at 6,000 rpm for 30 min to extract the enzyme, the supernatant was applied (Bruno et al.,2018).

Determination of prolidase activity

Alanin-proline (Ala- pro) is used to measure prolidase activity as a substrate and standard substrate for the enzyme assay. Take 100 ml from Ala-pro, 100 ml from Tris HCl and 100 ml from the enzyme and incubate at 37°C for ten min. The reaction was stopped by adding 1400 ml from 1M sodium acetate pH (2.8) buffer, 200 ml of the ninhydrin solution, added to the tube and mixed, then heated at 70°C for 10 min incubation. The yellowish color that developed due to free proline was detected by measuring 440 nm [6].

Optimal conditions for prolidase production

Prolidase produced by the selected isolate was determined via inoculating 100 ml of the minimal salt medium (pH 7) to 1 ml of fresh culture (OD =0.6) of E.coli and incubated at 37 ºC for 24 hrs. Prolidase activity and protein concentration in the crude cell-free supernatant were estimated. Optimum factors were determined as optimum nitrogen source (Peptone, Yeast extract, tryptone, casein, and meat); optimum carbon source (starch, sucrose, glucose, glycan and maltose); and optimum temperature (25, 30, 35, 40, 45, 50 ^{o}C).

Extraction of prolidase

Extraction and fractionation of prolidase was done by washing the bacterial cells twice with 10Mm Tris –HCl buffer (pH 8), re-suspending in 400ml of the same buffer, and incubating at 37 C^{o} for three hours. After that, high-speed centrifugation (15,000 x g for ten min.) was done, and cold acetone (175 ml) was

added. The mixture was centrifuged at 5000 x g for ten minutes to remove contaminating proteins as precipitated. Then, 455ml of cold acetone was added, and another centrifugation was performed to precipitate the enzyme under the same condition. Re-suspended this pellet in 10Mm Tris- HCl (pH 8), then left to evaporate the acetone and measured the enzyme activity and protein concentration [6].

Dialysis of crude enzyme

The precipitates of the crude prolidase were purified by dialysis bag with 8000 MW cutoff against 20mM Phosphate buffer saline at pH 7.2, incubated overnight against the Phosphate buffer at 4ºC and concentrated with sucrose (Li et al.,2016).

Purification via Ion exchange chromatography

According to [7] DEAE– the cellulose column (2×25 cm) was equilibrated previously with 0.05 M phosphate buffer pH 7. Each fraction measured the absorbance at 280 nm by using the UV-VIS spectrophotometer. Enzyme activity was measured for each fraction.

2.9. Purification via gel filtration chromatography

Sephadex G-150 in a glass column (2 × 40 cm) was used, and elution was achieved at a flow rate of 30ml /hr. The absorbance of each fraction was measured at 280nm. Enzyme activity in each fraction and protein concentration determined by using [8].

Characterization of the prolidase

Some of the characteristics of the purified prolidase were determined as follows:

Effect of pH on purified prolidase

The purified enzyme was added to 200ml of substrate solutions prepared at different pH varies ranging from (4-9), including acetate buffer PH (4, 4.5, 5), potassium phosphate buffer PH (6,6.5, 7,7.5), and Tris-HCl buffer PH (8, 8.5,9). The enzyme activity was assayed.

Optimal pH for purified prolidase stability

Buffer solutions at different pH values ranging between 4 -10 were mixed with purified enzyme and then incubated at 37 Cº for 30min. After that, it is directly cooled in an ice bath. Enzyme activity was measured, and remaining activity was calculated.

Effect of the temperature on prolidase activity

Determination of the prolidase activity after incubating the purified enzyme with the substrate at different temperatures of 30-50 ºC.

Effect of temperature on prolidase stability

The purified enzyme was incubated at different temperatures (30 -50 ºC) for 30 min, using the ice bath immediately. Enzyme activity was assayed at each temperature. The remaining activity (%) for prolidase was calculated and plotted against the temperature ºC.

Various ions and inhibitors affect prolidase determination

Different metal ions (equal volume) were incubated with enzyme ($CoCl_2$, $CaCl_2$, $MgCl_2$, $MnCl_2$, $CuCl_2$ and $FeCl_2$) 1M at 37 ºC for 30 minutes. Then, the enzyme activity for each treatment was measured. The control was the enzyme solution only. The remaining activity was assayed for each treatment.

Antibiofilm Formation

The microtiter plate method was used to test the ability of prolidase as an antibiofilm against pathogenic microorganisms [27]. An ELISA reader determined Optical Density, and antibiofilm was calculated as follows:

$$\text{Inhibition of Biofilm} = \frac{\text{O.D. in control} - \text{OD in treatment}}{\text{OD in control}} * 100$$

Prolidase cytotoxicity in vitro

According to [28], Brain Tumor Cell Line (A127) and Colorectal Adenocarcinoma cells (CaCo-2) were used in comparison to Normal embryonic liver cell line (WRL-68) by MTT assay to detect the inhibition concentration (IC50) values and viability cells when purified prolidase was used.

The A172, CaCo-2 and WRL68 cell lines were used in complete medium RPMI-1640. Incubated culture medium flasks in atmospheric 5% CO2 at 37°C. The obtained concentration of the cells using the trypan exclusion cell counting method and the cells were counted microscopically using a hemocytometer and applying the formula:

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

Result

Bacterial Isolates

In this study (100) samples were collected from the stool of infants breastfeeding, vomiting and non-diarrhea to isolate bacteria. The characteristics of Escherichia coli colonies appeared on nutrient agar circular, large, low convex and MacConkey agar; colonies were large pink colonies due to lactose fermentation.

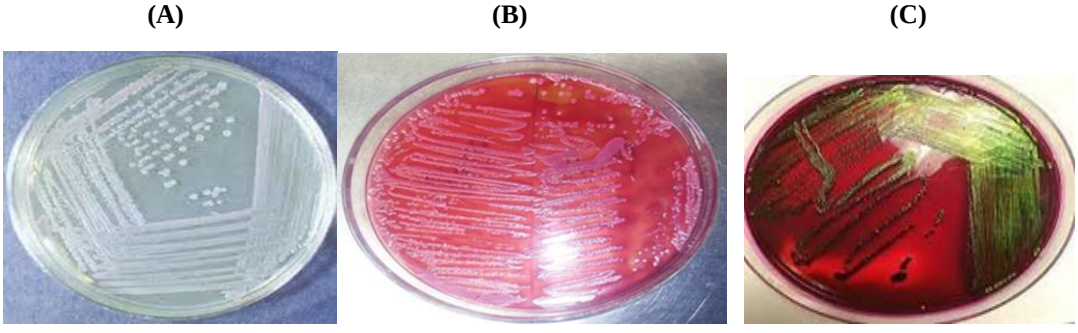

Figure 1. (A) *E. coli on* Nutrient agar. (B) *E .coli* on MaConkey agar. (C) *E. coli* on EMB.

Confirmation of identification of E. coli isolates

The gene of 16S ribosomal RNA was used to make a confirmation check for 100 isolates of E. coli isolation. Figure 2 shows the results of the polymerase Chain Reaction in the 16S rRNA gene (585 bp) found in all isolates of E. coli.

Molecular detection of the 16S rRNA gene via PCR is one of the most commonly used techniques for identifying E . coli.

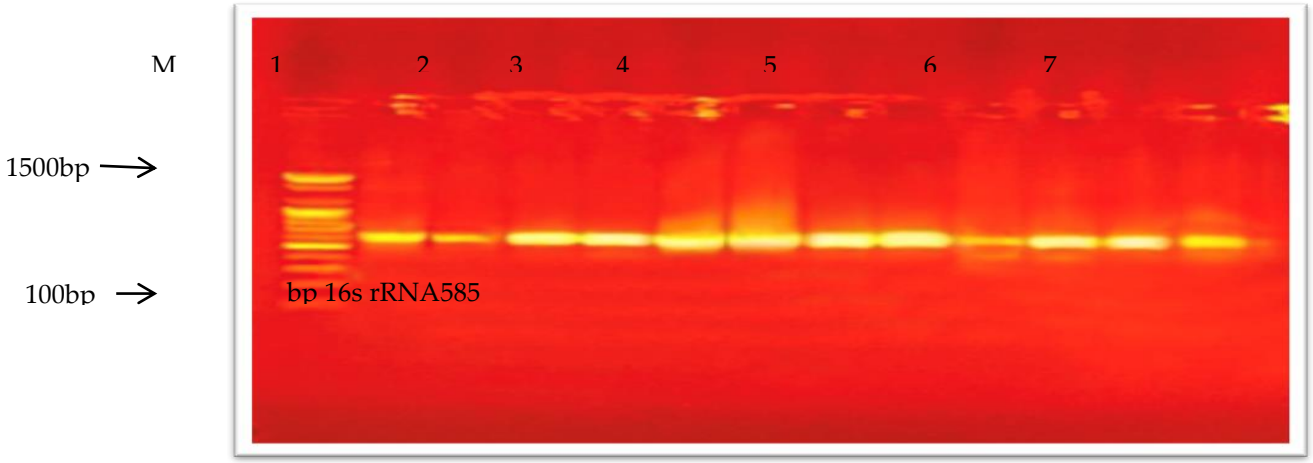

Figure 2. Agarose gel 2% electrophoresis of PCR amplified products 585 for 16S rRNA gene compared with (100bp) DNA ladder.

Screening of prolidase from E. coli

Semi-Quantitative analysis

Prolidase agar medium was used in semi-quantitative screening for prolidase production, and according to the results, there were strong, moderate and weak producers with clear zones, as shown in Figure 3. At the same time, negative isolates did not produce clear zones.

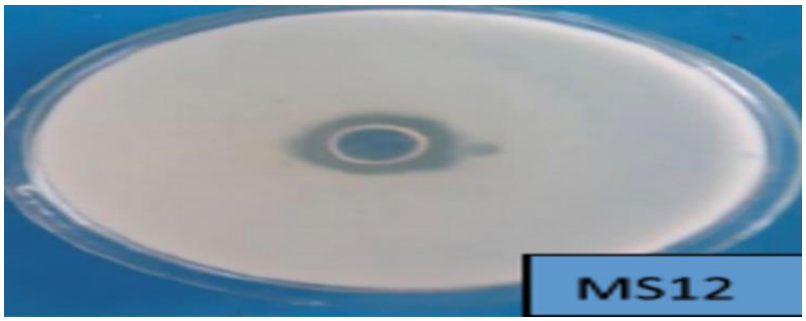

Figure 3. *E.coli* MS 12 producer isolate on prolidase agar medium.

Depending on the diameter of clear zones, only 40 (80%) isolates had the ability to produce prolidase from the (50) isolates, considered a non-pathogen. The diameter extended between (7-29) mm, and E.coli number (12) was the best and most intense producer of prolidase with a diameter of visible transparent zone equal (29 mm), so it was chosen for further experiments.

Quantitative analysis

According to the semi-quantitative method, 40 E. coli isolates could produce prolidase enzymes. However, in the quantitative method, we chose only 32 isolates out of these 40 isolates that gave a larger clear zone. These 32 isolates revealed different levels of prolidase production with specific activity equal to (2.1U/mg) via E.coli MS12, as shown in Figure 4.

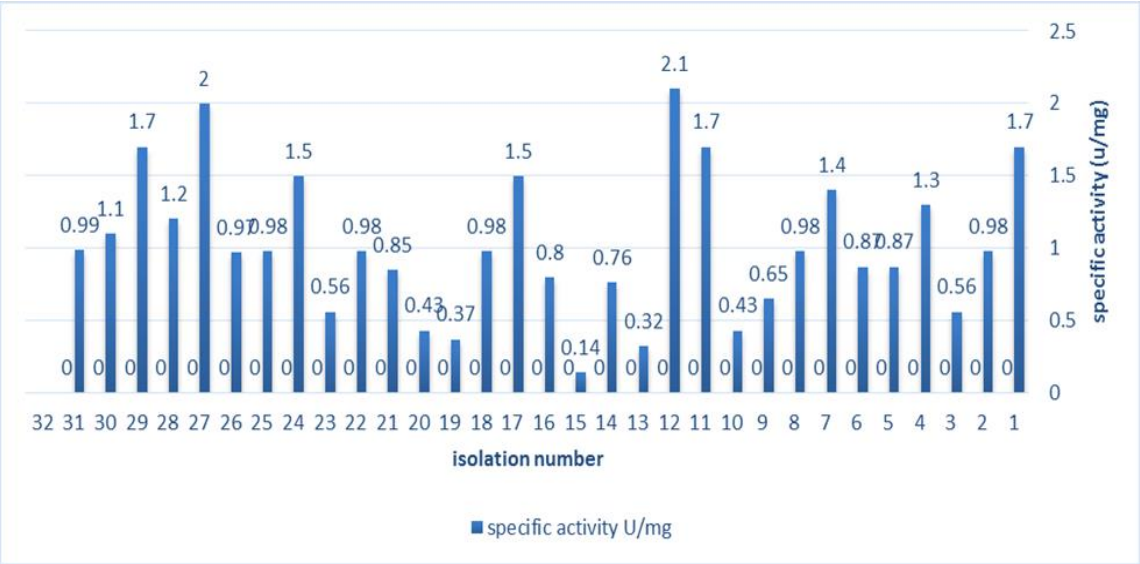

Figure 4. Specific activities for selected bacterial isolates in quantitative method.

Optimization of the growth conditions for prolidase production

Effect of Carbon Sources on the Production of Prolidase

Different carbon sources are used for determining the optimal carbon source for producing prolidase by E. coli MS12. The result showed in Figure 5 that among five types of carbon sources, sucrose was the best one and gave the higher prolidase-specific activity (2 U/mg). While the other types used in this experiment had a lower effect on the production of prolidase, and the lowest was starch (1.8 U/mg).

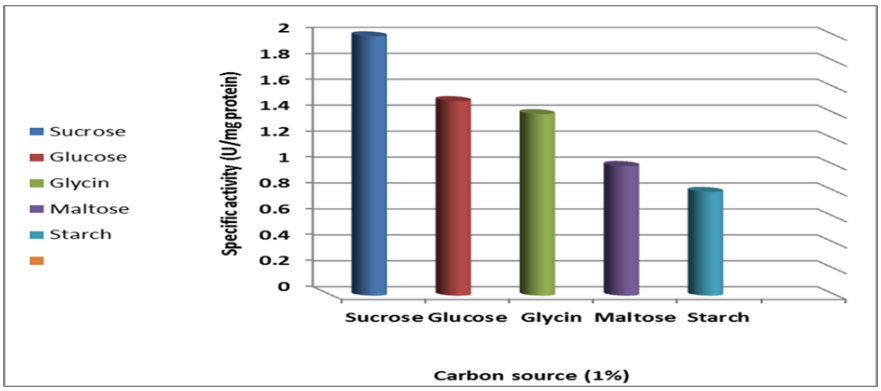

Figure 5. Effect of carbon source on prolidase production.

Effect of nitrogen sources

The effect of different nitrogen sources on prolidase production was carried out by using inorganic and organic sources, including (casein, meat extract, peptone, tryptone and yeast extract). The results showed that casein proved to be the best for prolidase production from E.coli, with a specific activity reaching (0.7 U/mg), followed via peptone, which was the second source of nitrogen suitable for prolidase production with a specific activity of (0.45 U/mg). In contrast, yeast extract, tryptone and meet extract represented the poor nitrogen sources for prolidase production, as shown in Figure 6.

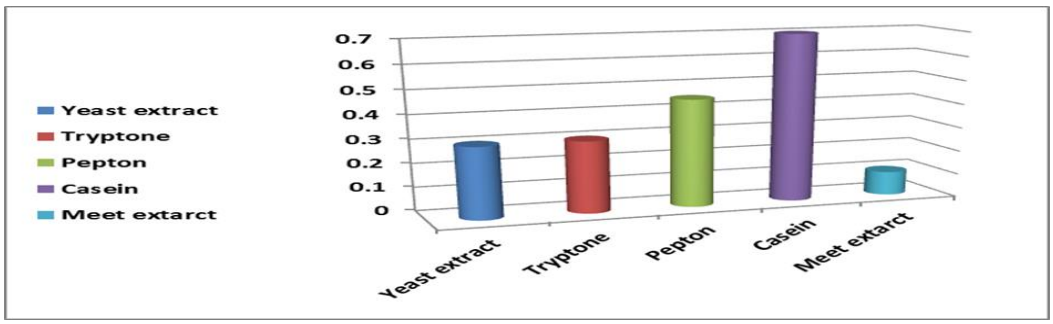

Figure 6. Prolidase production via *E.coli* MS 12 at different nitrogen sources.

Effect of incubation temperature

Different incubation temperatures (25°C - 45°C) were tested to determine the optimum for prolidase production by E. coli MS 12. Maximum Prolidase production appeared at 40°C, with specific activity (2 U/mg). While the specific activity decreased at all other range temperatures, as explained in Figure 7.

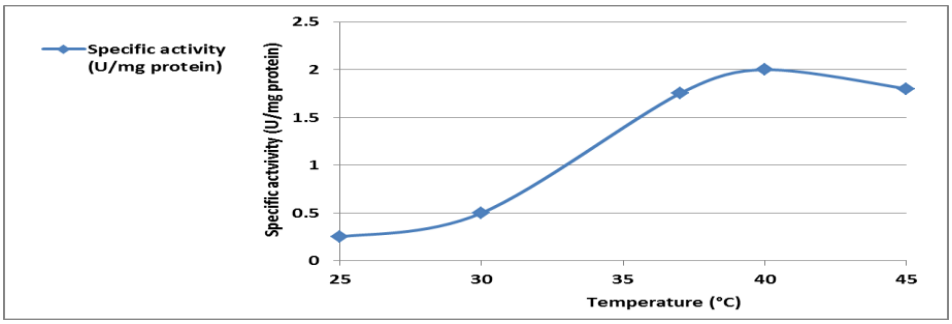

Figure 7. Effect of incubation temperature on prolidase production.

A study showed that E.coli isolates significant growth ($P<0.05$) when incubated at 40°C [18].

Extraction and purification of prolidase

After cold acetone precipitation and dialyzed, results showed that prolidase enzyme activity and specific activity were (2 U/ml) and (6.6 U/mg) protein, respectively.

Ion exchange chromatography (DEAE-Cellulose)

The DEAE-cellulose column was used in purification, and the result indicated that prolidase produced by E.coli MS12 has a negative net charge since it is bound with cationic ion exchange (DEAE-cellulose). Fractions were tested and pooled for prolidase activity with (1.5 U/ml), specific activity (15 U/mg), a fold purification of (7.5) and enzyme yield of (30 %) as shown in Figure 8 and Table 1.

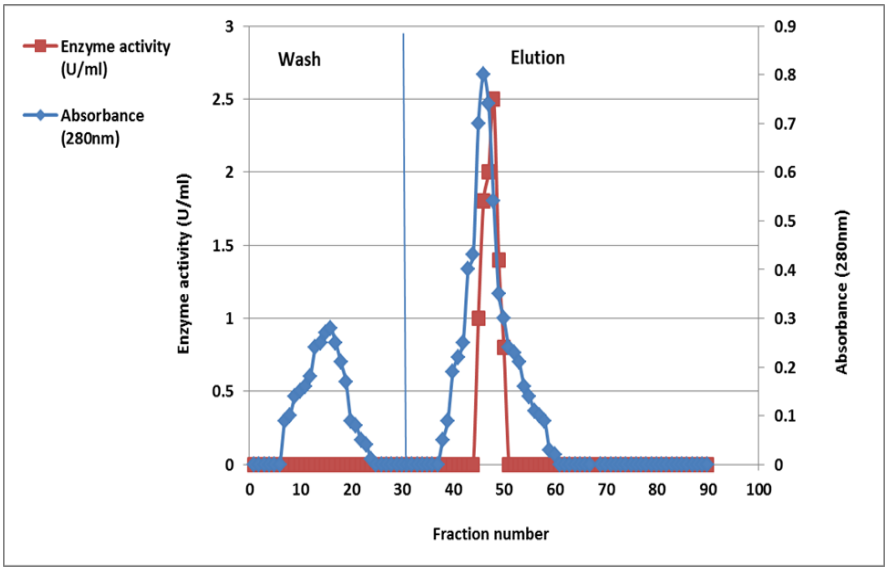

Figure 8. Ionic exchange chromatography for prolidase through DEAE cellulose column (1.5 x 17.5) cm. The column was calibrated with 0.1M acetate buffer pH5, flow rate 60ml/hrs and 3 ml fraction.

Gel filtration chromatography

Sephadex–G150 is used for further purification. The fractionation yielded one protein peak as absorbance, reading at 280 nm; this peak (fractionation tubes 7-35) contained prolidase activity (1 U/ml), specific activity (25 U/mg), and the purification fold was (12.5), with a yield of the enzyme (30%) as mentioned in figure 9 and table 1.

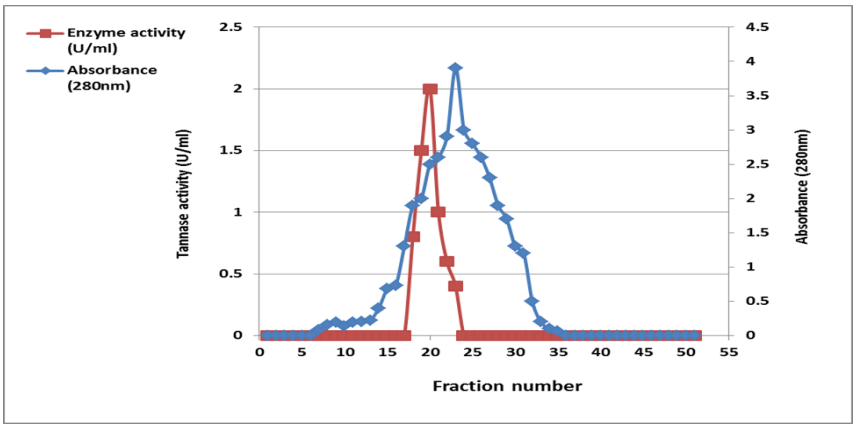

Figure 9. Gel filtration chromatography for purified prolidase from *E.coli* MS12 using Sephadex G-150 column (2×40) cm.

Purification step	Volume (ml)	Enzyme activity (U/ml)	Protein con-centration (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification (folds)	Yield (%)
Crude enzyme	75	0.8	0.4	2	60	1	100
Acetone pre-cipitation	25	2	0.3	6.6	50	3.3	83.3
Concentration	12	2.5	0.2	12.5	30	6..25	50

by saccharose							
DEAE-cellulose	**18**	**1.5**	**0.1**	**15**	**27**	**7.5**	**45**
Sephadex G150	**18**	**1**	**0.04**	**25**	**18**	**12.5**	**30**

Table 1. Purification steps of prolidase from *E. coli* MS12.

Characterization of purified prolidase

Effect of pH on Prolidase Activity

Prolidase activity purified from E.coli has been determined at variable pH (5, 6,7,8,9, and 10). Results revealed the optimal pH for purified prolidase activity at pH 7. However, prolidase activity was decreased at pH (5, 9and 10). While prolidase was still active over the range (6 to 8) of pH values, as shown in Figure 10.

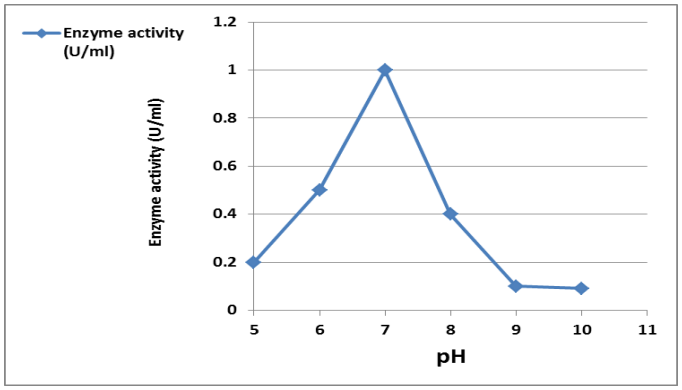

Figure 10. Effect of pH on prolidase activity from *E.coli* MS12.

Effect of pH on prolidase stability

Prolidase of E .coli was stable in a wide range of pH (5 to 10) and at pH (7 and 8) with remaining activity at 100%, while in pH6, the activity was 90%, as in Figure 11.

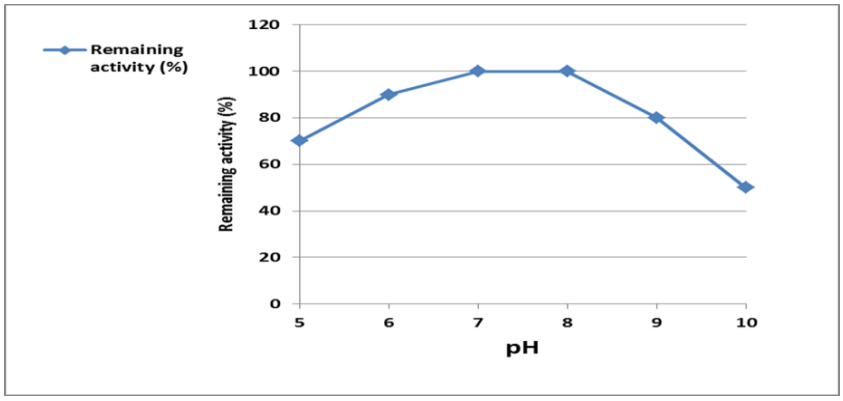

Figure 11. Effect of pH on prolidase stability from *E.coli* MS12.

Each enzyme has an optimal pH at which it catalyzes the reaction at its most excellent rate. Minor pH deviations from the optimum value decrease activity due to changes in the ionization of the groups at the enzyme's active site, whereas more significant pH deviations result in denaturation of the enzyme protein due

to interaction with several weak non-covalent bonds. In contrast, the three-dimensional structure of the enzyme is preserved [22].

Effect of temperature on prolidase activity

To determine the optimum temperature of prolidase activity, the enzyme reaction was carried out at different temperature degrees (30,35,40,45, and). Purified prolidase from E. coil MS12 showed the highest activity at 35°C Figure 12.

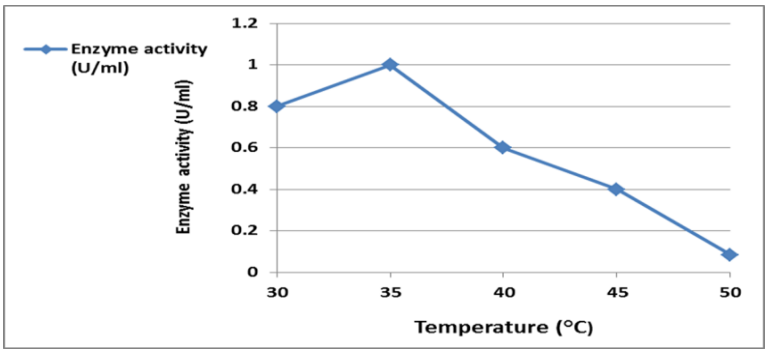

Figure 12. Effect of temperature on prolidase activity from *E.coli* MS12.

Effect of temperature on prolidase stability

The effect of temperature on prolidase stability was assayed in temperatures ranging from 30 to 80 C. The enzyme retained all its activity (100%) at temperatures from 40 -50°C, but it began to lose its activity beyond this temperature, Figure 13.

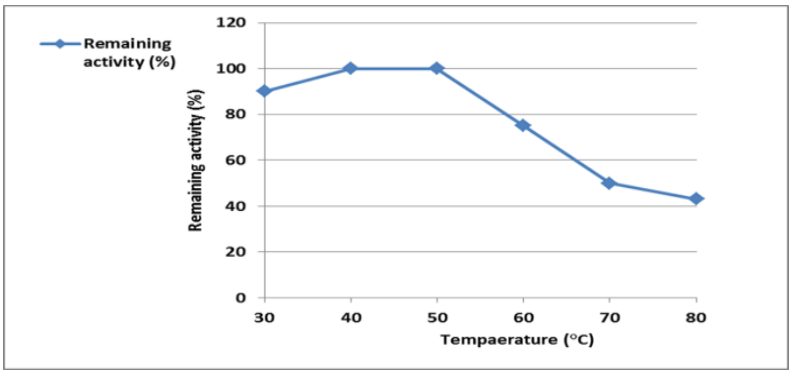

Figure 13. Effect of temperature on prolidase stability from *E.coli* MS12.

Effect of metal ions on the activity of the prolidase

Results in Table (2) illustrate the activity of prolidase. The higher enzyme activity (100%) when used FeCl2, CoCl2 and CuCl2, while when used CaCl2 and MnCl2 began to decrease to 80% and 65%, respectively, while no activity (0%) for MgCl2.

Reagent	Concentration (mM)	Remaining activity (%)
Control (Enzyme)		100
$FeCl_2$	1	100
$CaCl_2$	1	80

CoCl2	**1**	**100**
$CuCl_2$	**1**	**100**
$MnCl_2$	**1**	**65**
$MgCl_2$	**1**	**0**

Table 2. Metal ions affect prolidase enzyme activity.

Anti-biofilm activity of prolidase In vitro

Prolidase from E.coli MS12 was used at different concentrations (200, 300,400, 500 µg/ml). It showed the highest effect of the enzyme on biofilm inhibition at a concentration of 500 µg/ml against P. aeruginous and then E. coli and reached 65% and 60.3%, respectively, as in Table 3.

Bacteria	Percentage (%) of antibiofilm
Escherichia coli	60.3%
Pseudomonas aeruginosa	65%
Staphylococcus aureus	58.7%
Staphylococcus epidermidis	28.5%
Candida spp.	55.5%

Table 3. Anti-biofilm activity of prolidase *in vitro.*

Bacterial biofilms are multicellular accumulations of microorganisms encased in an extracellular polymeric substance (EPS) matrix comprising polysaccharides, proteins/enzymes, lipids, foreign DNA, and lysed cell detritus. There are five distinct stages in the establishment of a biofilm: (1) reversible attachment, (2) irreversible attachment, (3) maturation-1, (4) maturation-2, and (5) dispersion [24].

Cytotoxicity and Anticancer activity of prolidase

Brain Tumor Cell Line (A127), Colorectal Adenocarcinoma cells (CaCo-2) and Normal embryonic liver cell line(WRL-68) were used to test the prolidase effect on these cell lines. An assay of MTT was used to detect the inhibiter concentration (IC50) values of purified prolidase. Figure (14) showed that prolidase had a potent cytotoxic effect on the cancer cell line (A172) with 110µg /ml, while IC50 of regular cell line WRL-68 was 148.3 µg /ml.

The cytotoxic effect on the CaCo-2 cell line and IC50 was 99.34 µg /ml while IC50 of WRL-68 was 148.3µg/ml; this line gave more effect to the prolidase than A172 cells, as shown in Figure 15.

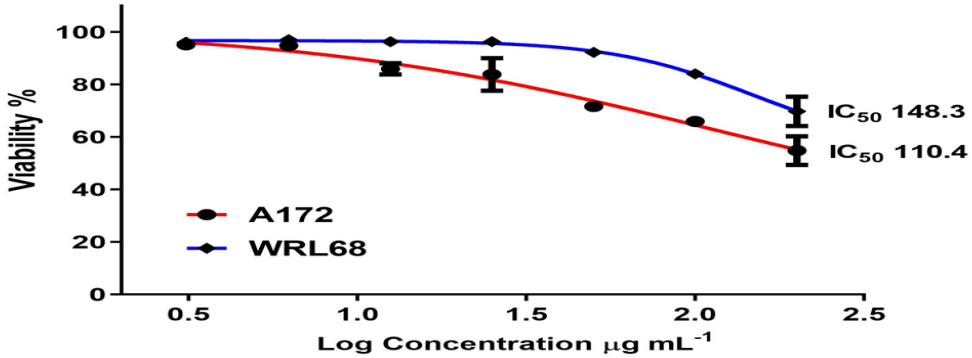

Figure 14. Cytotoxicity effect of prolidase on the viability of Brain Tumor Cell Line (A127) and Normal embryonic liver cell line (WRL-68).

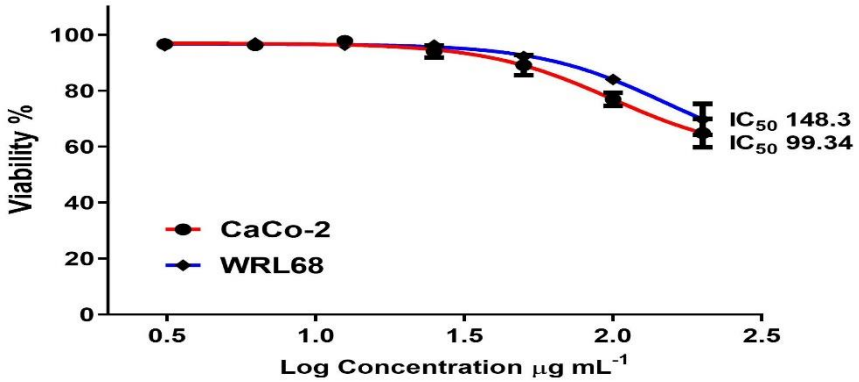

Figure 15. Cytotoxicity effect of prolidase on the viability of Human Breast Cancer Cells (CaCo-2) and Normal embryonic liver cell line (WRL-68).

Results of viability cells showed that the highest effect of prolidase on the cancer cell line (A172) at the concentrations 100 and 50 µg /ml was 65.90% and 71%, respectively. It is a significant comparison with normal cells (WRL-68), 84.03% and 92.28%, respectively, as shown in Table 4, while in 200 µg /ml, cell viability of A172 and WRL-68 does not have significance.

Concentration µg/ml	Cell viability (%) ± SD	
	A172	WRL 68
200.00	54.82 ± 5.46	69.81 ± 5.58
100.00	65.90 ± 1.77	84.03 ±1.25
50.00	71.64 ± 0.42	92.28 ± 100
25.00	83.84 ± 6.21	96.33 ± 0.41
12.50	86.00 ± 2.14	96.30 ± 0.87
6.25	94.79 ± 0.61	97.11 ± 1.04
3.10	95.18 ± 0.68	96.10 ± 0.88

Table 4. Viability ratio of A172 and WRL 68 cell lines by purified prolidase at different concentrations.

Also, at 100 µg/ml concentration, the prolidase showed the highest effect on the cancer cell line (CaCo-2) compared with the standard cell line (WRL-68), 76.93 % and 84.03%, respectively. However, the (200, 50, 25, 12.50, 6.25 and 3.10 µg/ml) are insignificant in Table 5.

Concentration µg/ml	Cell viability (%) ±SD	
	CaCo-2	WRL 68
200.00	64.89±5.11	69.81 ± 5.58
100.00	76.93± 2.34	84.03 ±1.25
50.00	89.16± 3.60	92.28 ± 1.00
25.00	94.06 ± 2.15	96.33 ± 0.41
12.50	97.84 ± 0.70	96.30 ± 0.87

6.25	**96.30 ± 1.10**	**97.11 ± 1.04**
3.10	**96.64 ± 0.53**	**96.10 ± 0.88**

Table 5. Viability ratio of CaCo-2 and WRL 68 cell lines by purified prolidase at different concentrations.

In agreement with the study, Proline dehydrogenase (PRODH) is up-regulated in evoke- pro-survival and cancer cells and activity, in vitro and in vivo [25]. [26] showed that breast cancer, pancreatic cancer, lung carcinoma, Stage 1 endometrial cancer, stomach cancer, and ovarian cancer have all been linked to increased serum prolidase activity.

Discussion

Green Metallic sheen colonies were formed on Eosin methylene blue agar because of lactose fermentation and production of solid acids, as in Figure 1. These features come by the corresponding cultural characteristics mentioned via [9,10,11], respectively. Microorganism identification must be precise and conclusive in a range of applications, including labor-intensive biochemical characterization or 16S rRNA sequencing to confirm species type [12]. [13] shows the apparent species-specific nature of 16S rRNAs, found that the E. coli ribosome can depend on 16S rRNA. Prolidase is an important enzyme, called proline dipeptidase, is one of these unique enzymes capable of degrading dipeptides in which a proline or hydroxyproline residue is located at the C-terminal position (Xaa-Pro) [2]. Protease enzyme produces a halo zone from Bacillus subtilis isolates at thermostable [14]. Prolidase's best-researched substrates are imidodipeptides, which are formed during the degradation of collagen, the most abundant component of the extracellular matrix (ECM) [2].[1] Required enzyme prolidase in several pathological and physiological processes like inflammation, wound healing and angiogenesis. In agreement with this study, researchers found that sucrose was to be the optimal initial carbon at the optimal concentration using a series of fed-batch cultures of E. coli [15]. Sixteen shown in the study, the highest enzyme activity of L-asparaginase from E. coli was lactose and tryptone. Consider that the nitrogen source is the primary nutrient after the carbon essential to the microorganisms for growth. The parts needed for nitrogen is nucleotides, protein, cofactors and enzyme, which have a vital role in metabolism [17]. Purification of prolidase from the serum of patients with polycystic ovarian syndrome utilizing Gel filtering technology and Sephadex G100 gel as a stationary phase was also discovered (0.00176 IU/I) specific activity [19]. Escherichia coli produces prolidase, a particular exopeptidase. By fractionated ammonium sulfate precipitation, ion exchange chromatography on DEAE-Sephadex A 50, and gel filtering on Sepharose 4 B, with 67-fold enrichment, the total yield of prolidase is 19 percent [20]. Twenty-one were shown prolidase activity increased and reached a maximum at pH 8, while the immobilized prolidase enzyme shifted to pH 8.4. Metal ions can bind to proteins and form complexes with other molecules connected to enzymes, functioning as electron suppliers or acceptors or as structural regulators and regulators. By interacting with the amine or carboxylic acid groups of amino acids, these ions can activate or inhibit enzyme activity [23].

Conclusion

As far as we know, this study is the first to investigate the role of prolidase enzyme (which is purified from E.coli bacteria) in the elimination of biofilm formed by pathogens and its sound effect on two cancer cell lines (colon and Brain cancer cell line) in comparison with the standard cell line. So, enzyme

technologies are increasingly used in pharmaceutical research, development, and manufacturing.

Acknowledgment: The author would like to thank Al-Mustansiriyah University, Baghdad, Iraq (www.uomustansiriyah.edu.iq) for the support of this work.

References

1. Ireti E., Zeljka M. L., Muthukumar B., Chandravanu D., and Jui P. PROLIDASE: A Review from Discovery to its Role in Health and Disease. Front. Mol. Biosci. **2021**. https://doi.org/10.3389/fmolb.2021.723003.
2. Kitchener, R. L., and Grunden, A. M. Prolidase Function in Proline Metabolism and its Medical and Biotechnological Applications. J. Appl. *Microbiol*. **2012**; *113 (2)*, 233–247.
3. Vyas N.K., Nickitenko A., Rastogi V.K., Shah S.S., Quiocho F.A . Structural insights into the dual activities of the nerve agent degrading organophosphate hydrolase/prolidase. *Biochemistry*. **2010**; *49*: 547–559.
4. Tenaillon O, Skurnik D, Picard B, Denamur E (March). "The population genetics of commensal Escherichia coli". Nature Reviews. *Microbiology*. **2010**; *8 (3)*: 207–17.
5. Lowther, W.T. and Matthews, B.W. Metalloaminopeptidases: common functional themes in disparate structural surroundings. *Chem Rev.* **2002**; *102*, 4581– 4608.
6. Fujii, M.Nagaoka , Y.Imamura ,SH. and Shimizu ,T. Purification and Characterization of a Prolidase from Aureobabacterium esteraromaticum, Bioscience, *Biotechnology, and Biochemistry*, **1996**; *60:7*, 1118-1122.
7. Whitaker .J.R.and Bernard, R.A. Experimned for introduction to Enzymology.The Wiber Press Davis **1972**.
8. Bradford, M. M. A rapid and sensitive method for quantitating microgram quantities of protein utilizing the principle of protein–dye binding. Aral. *Biochem*. **1976**;*72*: 248-49.
9. Ojeda, R. JS Improved tannase production by E. cloaca using Indian gooseberry leaves under submerged fermentation using Taguchi approach. *J. of AMB Expr*., **2017**; *6(1)*: 46.
10. Karen Elbing and Roger Brent. Recipes and tools for the culture of Escherichia coli. *Curr Protoc Mol Biol. Jan*; **2019**, *125(1)*: e83.
11. Sagar, A. EMB Agar- Composition, Principle, Preparation, Results, https://microbenotes.com/eosin-methylene-blue-emb-agar. **2022**.
12. Mohammed, S.M., Anup K., Anil K. R., and Shipra S. Identification of Escherichia coli through analysis of 16S rRNA and 16S-23S rRNA internal transcribed spacer region sequences. *Bioinformation*. **2011**; *6(10)*: 370–371.
13. Kitahara, K., Yasutake, Y., and Miyazaki, K. Mutational robustness of 16S ribosomal RNA, shown by experimental horizontal gene transfer in Escherichia coli. *Proc. Natl. Acad. Sci. U.S.A*. **2012**; *109*, 19220–19225.
14. Yago Q., Katharina M. D., Gabriela C. C., Elizeu A. Screening of protease, cellulase, amylase and xylanase from the salt-tolerant and thermostable marine Bacillus subtilis strain SR60 [version 1; peer review: 1 approved with reservations, 1 not approved].7:1704 last updated:25 NOV2020. **2018**.
15. Ning C., Jin H., Zhi-bin F., Lei Y., Qing-yang X. & Ting-yi W. Optimization of Fermentation Conditions for the Biosynthesis of L-Threonine by Escherichia coli. Applied Biochemistry and Biotechnology volume 158, pages–604. **2009**.
16. Seyedeh, L. and Iran, A. Production of l-asparaginase from Escherichia coli ATCC 11303: *Optimization by response surface methodology*. **2011**;*89*, 4, 315-321.
17. Suribabu, K., and Hemalatha, K.P.J. Thermal Optimization of α-amylase Production in Brevibacillus sp. *Int. J. Curr. Microbiol.App.Sci*., **2016**; *5(3)*: 909-16.
18. Kim C., Wilkins K., Bowers M., Wynn C., and Ndegwa E. Influence of PH and Temperature on Growth Characteristics of Leading Foodborne Pathogens in a *Laboratory Medium and Select Food Beverages*. **2018**;*3(1)*:1031.
19. Remy M. Mohamed Saleh, Firas T . Maher and Nagham Q. Kadhim. Study of some hormones and Partial purification of prolidase from the serum of women with polycystic ovary syndrome. *Tikrit* , **2019**;*24 (7)*.

20. Ruttloff H., Friese , E. , Lasch, J.and Koelsch, R. Gewinnung und Charakterisierung von Prolidase aus Escherichia coli B, II. Charakterisierung, Reinigung und Trägerfixierung des Enzyms. *Engendering*. **1982**; *2(2)*:161-170.
21. Tzu-Fan W., Meng-Chun C., Kuan-ling L., Min-Guan L., Yi-Yu C., Huei-Fen L. and Long-Liu L. High-level expression and molecular Characterization of recombinant prolidase from Escherichia coli NovaBlue. PeerJ 6:e5863; DOI 10.7717/peerj.5863. **2018**.
22. Ibrahim, R. A. Purification, Characterization and cytotoxic activity of arginine deiminase from Pseudomonas aeruginosa clinical isolate. Master thesis, College of Science, Al-Nahrain University, IRAQ. **2018**.
23. De Cassia Pereira, J.; Giese, E. C.; de Souza Moretti, M. M.; dos Santos Gomes, A. C.; Perrone, O. M.; Boscolo, M. and Martins, D. A. B. Effect of metal ions, chemical agents and organic compounds on lignocellulolytic enzymes activities. *Enzyme Inhibitors and Activators,* **2017**;*29*, 139-164.
24. Rybtke, M., Hultqvist, L. D., Givskov, M., & Tolker-Nielsen, T. Pseudomonas aeruginosa biofilm infections: community structure, antimicrobial tolerance and immune response. *Journal of molecular biology*; **2015**; *427(23)*, 3628-3645.
25. Phang JM, Liu W, Hancock C, Christian KJ. The proline regulatory axis and cancer. Front Oncol 2:60. **2012**.
26. Arioz, D. T., Camuzcuoglu, H., Toy, H., Kurt, S., Celik, H., and Aksoy, N. Serum Prolidase Activity and Oxidative Status in Patients with Stage I Endometrial Cancer. Int. J. Gynecol. *Cancer*, **2009**; *19 (7)*, 1244–1247.
27. Babapour, E.; Haddadi, A.; Mirnejad, R.; Angaji, S-A. & Amirmozafari, N. Biofilm formation in clinical isolates of nosocomial Acinetobacter baumannii and its relationship with multi-drug resistance. *Asian Pacific Journal of Tropical Biomedicine*. **2016**; *6(6)*: 528–533.
28. Freshney, R. I. Culture of animal cells: a manual of basic technique and specialized applications, John Wiley and Sons. **2015**.
29. Suardana I W. Analysis of Nucleotide Sequences of the 16S rRNA Gene of Novel Escherichia coli Strains Isolated from Feces of Human and Bali Cattle. J Nucleic Acids. 475754. **2014**.
30. Tille, P.M. Baily and Scott's Diagnostic Microbiology. 14th ed. Mosby Elsevire Inc. China. **2017**.
31. Viglio, S., Annovazzi, L., Conti, B., Genta, I., Perugini, P., Zanone, C., Casado, B., Cetta, G. et al. The role of emerging techniques in investigating prolidase deficiency: from diagnosis to developing a possible therapeutical approach. *J Chromatogr B Analyt Technol Biomed Life Sci*. **2006**;*832*, 1–8.

Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation: Abdul Hussein, S.A.; Zaki, N.H.; Rasool, KH Biological activities of purified prolidase from pathogenic *E.coli.* Revis Bionatura 2023;8 (1) 57. http://dx.doi.org/10.21931/RB/CSS/2023.08.01.57