Purification of Alkaline Protease from Hydrolyzed Chicken Feather Waste Using Recombinant *B. Subtilis* Strain

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Abstract:

Extracellular alkaline protease was produced during aerobic cultivation of *Bacillus subtilis* DB100 (pS1) in a medium containing 2% chicken feather waste (CFW) for 48 hrs. The optimal pH and temperature of alkaline protease were 7.2 and 45°C respectively. The crude enzyme solution was precipitated with solid $(NH_4)_2SO_4$ (65% saturation) and dialyzed against 0.1 M Tris-HCl, pH 7.5 containing 10 mM $CaCl_2$. Purification was carried out using DE-52 ion exchanger column followed by gel filtration in Sephadex G-50 column. The protein content in fractions of DE-52 ion exchange & Sephadex G-50 was detected by measuring absorbance at 280 nm. The activity of alkaline protease was detected in the same fraction by its specific methods. After dialysis, about 91% of the alkaline protease total units were retained with 18.7 fold purification. After DE-52 column, about 84.6 % of the alkaline protease total units were retained and about 56 fold purification was achieved. After Sephadex G-50 column, about 68.5% of the enzyme total units were retained and about 75.5 fold purification was obtained. SDS – PAGE was carried out to check the purity of the alkaline protease enzyme sample. The resulted single protein band indicated that the enzyme was almost purified and corresponds to a molecular weight about 27 KDa.

Key words: Feather degrading bacteria, keratin, protease purification

Introduction:

Environmental wastes are found in great quantities everywhere. They occupy considerable space and are considered to be a real source for pollution and health hazards since various types of microorganisms, including pathogens, have the ability to grow on such wastes. Chicken feather waste (CFW) is one of these environmental wastes. It is mainly keratin and represents 5 to 7% of the total weight of mature chickens. It is also generated in large quantities as a by- product of commercial poultry processing (Riffel and Brandelli (2006) and Savitha *et al*. (2007)). Keratin, in its native state, is not degradable by common proteolytic enzymes such as trypsin, pepsin, and papain because of the high degree of cross linking by cysteine disulphide bonds, hydrogen bonding, and hydrophobic interactions

(Lin *et al*. (1996) and Ali *et al*. (2011)). The chemical contents of feather, as percentage of dry weight are protein 85.4%; fats 1.22 %; Ash 8.60 %; fibers 0.68%; calcium 0.55%; and phosphorus 0.16% (Han and Parsons (1991) and Riffel and Brandelli (2006)).

Proteases are among the most important class of industrial enzymes, which constitute more than 65% of the total industrial applications, such as laundry detergent, leather preparation, meat tenderization, peptide synthesis, food industry, de-hairing process, pharmaceutical industry; as well as in bioremediation process. Proteases are also used in textile industry for removing the stiff and dull gum layer of sericine from the raw silk fiber leading to its brightness and softness. An interesting role in the decomposition of gelatinous coating of X-ray films from which silver was recovered (Anwar and Saleemuddin (1998) and El-Shafei *et al.* (2010)). The keratinolytic microorganisms and technologies developed for feather degradation not only remove the waste feathers efficiently from the nature but also make the by-products of the process as a value protein supplement. The protein rich, concentrated feather meal can also be used for organic farming as nitrogen fertilizer (Kainoor and Naik, 2010). Several amino acids, soluble proteins and peptides and mainly proteolytic enzymes were produced earlier from growing of *B. subtilis* DB100 (pS1) on CFW (Zaghloul *et al.*, 2004). The present study was achieved to produce and purify alkaline protease hydrolyzed CFW using recombinant *B. subtilis* DB100 (pS1) strain.

Materials and Methods:

Bacterial strain and plasmid:

B. subtilis DB100 (pS1) strain was used in this study. *B. subtilis* DB100 (pS1) cells harbors the multicopy (pS1) plasmid. The pS1 plasmid is a pUB110 derivative plasmid (6.7 kbp) that was constructed earlier by Zaghloul (1986). It carries the complete *B. subtilis* alkaline protease (apr A) gene as well as a kanamycin resistance marker. The complete aprA gene is a part of the Sma1/ Pst1 (2.7 Kbp) fragment. In *B. subtilis* cells, pS1 replicates at a level of 50 copies per chromosome (Zaghloul *et al.* (1986) and (Zaghloul (2004)).

Media:

PY medium (Peptone Yeast Extract medium) contained 10 g peptone, 5 g yeast extract, and 5 g NaCl per liter. The pH was adjusted to pH 7.0 (Bernhard *et al.*, 1978). PA kanamycin medium (Peptone Yeast Extract Agar medium) is PY supplemented with 15 g agar per liter containing 5µg kanamycin per ml medium. Basal medium II (NH_4Cl, 0.5 g; NaCl, 0.5 g;

K_2HPO_4 , 0.3 g; KH_2PO_4 , 0.4 g; $MgCl_2$. $6H_2O$, 0.1 g; and yeast extract, 0.1 g per liter), pH 7.0. Physiological saline solution contained 8.5 g NaCl per liter. All media were autoclaved at 115° C (Pelczar and Chan, 1977).

Determination of viable count:

One ml of the bacterial culture was taken and diluted with physiological saline. Out of each dilution, 100 µl was placed on PA kanamycin plates in which the bacterial colonies (colony forming units, CFU/ml) were counted (Pelczar and Chan, 1977).

Determination of the alkaline protease activity:

The activity of the alkaline protease was determined according to the methods of Lin *et.al,* (1992). Reaction mixtures (2 ml) contained 10 mg casein, 200 µmole sodium carbonate buffers, pH 9.7 and 0.1 ml of the enzyme. The reaction was carried out at 37° C for 30 minutes, and then terminated by the addition of 3 ml, 5% (w/v) trichloroacetic acid (TCA), and 3.3 M HCl. The reaction was then kept on ice for 1hour after which they centrifuged for 30 minutes at 4,000 rpm. The absorbance of the TCA soluble mixture was measured at 280 nm. The activity of the alkaline protease was also determined according to the method of Lin *et.al.* (1992). One gm of skim milk was dissolved in 100 ml 20 mM Tris - HCl buffer,(pH7.5) and 1.5gm agar was added. After heating, the mixture was poured in Petri dishes. Holes were pinched to accommodate 25 µl of the enzyme. The plates were incubated overnight at 37° C after which the clear zone around each hole was measured.

Determination of NH_2 - free amino groups using ninhydrin reagent:

In another attempt to check the activity of alkaline protease and choose the time which gives the maximum activity, NH_2-free amino groups were determined using ninhydrin as described earlier (Pearce *et al.,* 1988). Ninhydrin reagent was added at 300 µl to 300 µl of supernatant contained amino acids in 4M acetate buffer, pH 5.5. The above reaction tube were vortexed well then boiled in a water bath for 20 minutes. After cooling on ice, 400 µl of 50% (v/v) ethanol was added to each tube and the absorbance at 570 nm was measured after 10 minutes. NH_2- free amino groups' concentrations were calculated via a standard curve using the amino acid leucine.

SDS - polyacrylamide gel electrophoresis (SDS–PAGE):

SDS-PAGE was performed as described earlier by Laemeli (1970). Samples were prepared by mixing 100 µg protein samples with 2X sample

application buffer [0.6 M Tris - HCl, pH 6.8, 1% SDS, 10% β-mercaptoethanol, 10% glycerol, and 0.05% bromophenol blue] then boiled at 95° C for 3 minutes. Samples were applied to the 10 % gel along with SDS molecular weight marker (6.5-205 K.Da, Sigma, USA). Electrophoresis was carried out at a constant current 15 mA for about 1.5 hrs. The gel was then stained with Coomassie blue for two hrs. with agitation at room temperature followed with destaining.

Purification of the alkaline protease enzyme:

The recombinant *B. subtilis* DB100 (pS1) cells were grown on 2% CFW in Basal medium II for 48 hrs at 45°C with agitation as described before with some modifications (Upton and Fogarty (1977), Matsuzawa *et al.* (1988), and Sutar *et al*. (1991)). Cell-free supernatant was obtained by precipitating cells at 8,000 rpm. Solid ammonium sulfate was added to cell-free supernatant to reach 65% saturation. The precipitate was removed by centrifugation at 12,000 rpm for 30 minutes at 4°C. Pellets were resuspended in 0.1 M Tris-HCl buffer, pH 7.5 containing 10 mM $CaCl_2$, and dialyzed overnight against the same buffer. Samples were then taken to determine protein content and proteolytic activity. The alkaline protease was further purified using anion exchange DE-52 column. DE-52 anion exchanger 15g was suspended in excess of buffer A (50 mM Tris- HCl, pH 7.5) to be swelled. The slurry was poured into a (2.7 ×6 cm) column. The column was then washed and equilibrated with buffer A. Ten ml of the dialyzed alkaline protease was applied to the DE-52 column. The enzyme was eluted with 50 mM Tris-HCl buffer, pH 7.5 then 50 mM Tris-HCl buffer, pH 7.5 containing 0.5 M NaCl respectively at a flow rate of 36 ml/hr. Fractions (6ml) were collected after which the absorbance at 280 nm and the enzyme activity were determined. Active fractions were re-precipitated with solid $(NH_4)_2SO_4$ (65%) saturation, as mentioned above. The enzyme was purified further through Sephadex G-50 gel filtration column. Five g Sephadex was suspended in excess of buffer B (50 mM Tris-HCl, pH 7.5 containing 10 mM $CaCl_2$) to be swelled. The slurry was poured into (1.5 × 30cm) column. The column was then washed and equilibrated with buffer B. The dialyzed alkaline protease (2.2 ml) was applied to the Sephadex G-50 column. The enzyme was eluted with 50m M Tris-HCl buffer, pH7.5 containing 10 mM $CaCl_2$ at a flow rate of 36 ml/hr. Fractions (3 ml) were collected at 4°C after which the absorbance at 280 nm and the enzyme activity were determined. Active fractions were collected and re-precipitated on ice with solid $(NH_4)_2 SO_4$.

Protein determination

The protein contents were determined according to Lowry *et al.* (1951). Briefly, 400 µl of crude protein sample was added to 0.5 ml protein assay solution (5 % (w/v) Na_2CO_3, 1% (w/v) $CuSO_4$ and 2 % (w/v) sodium potassium tartarate). The tubes were mixed and allowed to stand for 10 minutes at room temperature after which 100 µl, 2 N Folin reagents was added. After 30 minutes, the developed color was measured at 750 nm. A standard curve was established using bovine serum albumin (BSA).

Results and Discussion:

Bacterial culture optimization:

To obtain the maximum activity, the extracellular alkaline protease was obtained from basal medium II supplemented with 2% CFW, 0.5 % corn oil, 0.1% yeast extract, pretreated with NaOH, directed with 5% *B. subtilis* DB100 (pS1) cells and grown at 45°C for 3 days. The biodegradation process started at a low rate then this rate was gradually increased. At day 2, the alkaline protease activity of the culture reached to the maximum activity when monitored using milk agarose plate assay (Fig.1) and casein method**.**

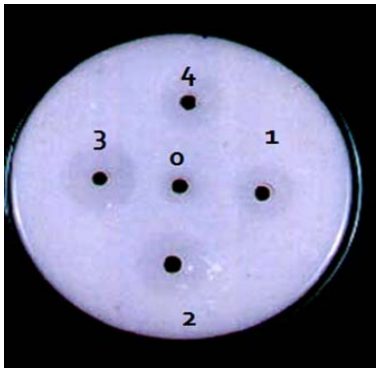

Fig.(1): The activity of alkaline protease enzyme using milk/agarose plate assay technique resulted from the *B. subtilis* DB100 (pS1) culture at day 0 - 4

The level of NH_2-free amino groups was monitored for the same culture. The results were compatible with those of enzyme activity. In general, the maximum alkaline protease activity (at day 2) and NH_2-free amino groups (at day 3) were 110 units/ml and 150 µmole leu/ml respectively (Table 1).

Table (1)
Monitoring the level of alkaline protease and NH2 - free amino groups during the utilization of 2% CFW directed by recombinant *B. subtilis* DB100 (pS1) cells

Cultivation time (Days)	CFU/ ml	(NH_2) free amino groups mole leu/ml µ	Alkaline protease Units / ml
0	$10^6 \times 7.9$	50	4
1	$10^8 \times 1.4$	95	44
2	$10^8 \times 1.5$	100	110
3	$10^9 \times 1.0$	150	76

Purification of alkaline protease enzyme:

The alkaline Protease was produced under the optimum conditions (2% CFW pretreated with NaOH, autoclaved two times, directed by recombinant *B. subtilis* DB100 (pS1) cells (5% inoculum) in cultures supplemented with 0.1 % yeast extract and 0.5 % corn oil, and incubated at 45 °C according to Zaghloul (1986)). The fractionation pattern and enzyme activity of the pre-dialyzed enzyme sample (10ml) using DE-52 ion exchanger column is shown in (Fig.2).

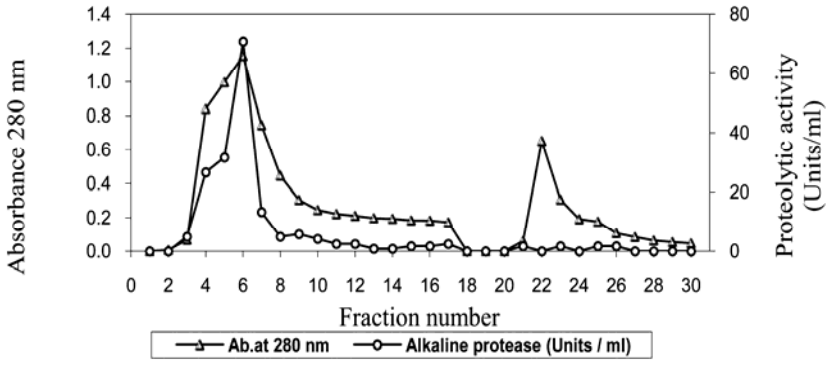

Fig.(2): Fractionation pattern and alkaline protease activity upon using DE-52 ion exchange column.

The pattern illustrated that two peaks of protein content (absorbance at 280 nm) were present. The larger peak (fractions 2–18) contained most of the alkaline protease activity, with a maximum activity of 70.8 units/ml.

The smaller protein peak (fractions 21-30) contained proteins other than alkaline protease. The enzyme was further purified using gel filtration Sephadex G-50 column. The fractionation pattern of this step is shown in Fig. (3).

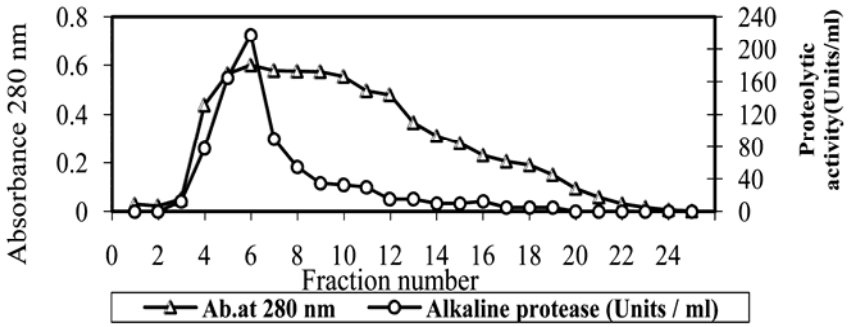

Fig. (3): Fractionation pattern of alkaline protease enzyme upon using gel filtration Sephadex G-50 column.

The pattern illustrated that, one peak of protein content (Ab.280) overlapped to a great degree with a single peak of proteolytic activity with a maximum activity of 217.5 units/ml (Fig.3). Results would indicate that, most of the protein (under this peak) is nearly in a pure form (alkaline protease). Data of the purification table (Table 2) illustrates that about 91% of the alkaline protease total units were retained after precipitation with solid $(NH_4)_2SO_4$ and dialysis with 18.7 fold purification was obtained.

After DE-52 ion exchanger column, about 84.6 % of the alkaline protease total units were retained with 56 fold purification was achieved. After Sephadex G-50 gel filtration column, about 68.5% of the enzyme total units were retained and 75.5 folds purification was obtained. The present study proved that the recombinant bacterial strain gave higher enzyme activity and fold purification than wild types or fungal strains (Porres *et al.* (2002), Gradišar *et al*. (2005), Cai *et al.* (2008), and Ali *et al.* (2011)). In addition, it saved time, cost and effort so it can be used much well in the industries.

Table (2)
Purification table of alkaline protease enzyme produced by the recombinant *B. subtilis* DB100 (pS1) cells when grown on basal medium II supplemented with 2% CFW, 0.5 % corn oil, 0.1% yeast extract, pretreated with NaOH and grown at 45°C for 3 days

Purification step	Volume (ml)	mg protein/ml	Units/ ml	Total units	Sp. Activity	Fold Purification	Recovery (%)
cell- free supernatant	700	14.5	71	49,700	4.89	1	100%
pellet after dialysis	29	17	1,560	45,240	91.76	18.76	91%
DE-52 column	6	8	2,200	13,200	275	56.23	84.6%
Sephadex G-50 column	2.2	5.2	1,920	4,224	369.2	75.5	68.5%

(1) One unit of alkaline protease is the amount of enzyme that produces one micromole of tyrosine at 37°C per 30 minutes using casein as a substrate.
(2) Fold purification was derived by dividing specific activity at any step by the specific activity at the initial step
(3) Recovery was derived by dividing total units at any step by the total units for the previous step.

SDS- polyacrylamide gel electrophoresis was carried out to check the purity of the alkaline protease enzyme sample and determination of its molecular weight. About 100 µg protein samples from cell-free supernatant, enzyme after DE-52 column, and enzyme after Sephadex G-50 column were applied separately to 10% SDS gel. Cell-free supernatant showed several protein bands while one single protein band was present in both samples after DE-52 and after Sephadex G-50. The resulted single band after Sephadex G-50 column indicated that the enzyme was almost purified and corresponds to a molecular weight about 27 kilodalton as compared to the molecular weight marker (6.5-205 K.Da) (Fig.4 and Table 3). This result was agreed with the previous studies where they reported that alkaline protease molecular weight is ranged from 25-31 KDa (Porres *et al.* (2002), Gradišar *et al*. (2005), and Cai *et al.* (2008)).

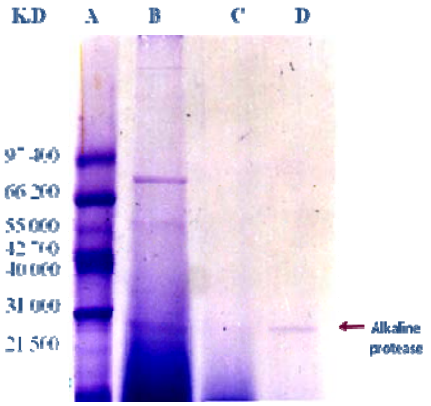

Fig. (4): SDS-PAGE of alkaline protease resulted from Biodegradation of CFW (A) molecular weight marker (6.5-205 K. Da), (B) cell free supernatant, (C) alkaline protease after DE-52 column, (D) alkaline protease after Sephadex G-50 column.

Table (3):
Relation between molecular weight of standard proteins (Sigma) as well as purified alkaline protease and their relative motilities when applied to 10% SDS-PAGE.

Molecular weight standard proteins	M.wt (Dalton)	(RF*)/cm
Phosphorylase, Rabbit Muscle	97,400	0.320
Albumin, Bovine Serum	66,200	0.430
Glutamic Dehydrogenase, Bovine liver	55,000	0.510
Ovalbumin	42,700	0.588
Aldolase	40,000	0.622
Carbonic anhydrase	31,000	0.750
Trypsin Inhibitor, Soybeen	21,500	0.840
Purified alkaline protease	27,000	0.700

* RF is the mobility of a given protein with regard to the mobility of the dye

Conclusion:

The *B. subtilis* DB100 (pS1) degraded feathers quickly, and the enzyme produced by this strain has high keratinolytic activity. The enzyme has a molecular mass of 27 kDa. The enzyme after purification showed the highest activity, fold purification and recovery percentage. This enzyme can

promote several industries with high efficiency such as meat tenderization, dairy industry, Pharmaceutical industry, Tanning of leather, Biodetergent, Bioremediation and amino acids industry.

Future Prospective:

Studying the kinetics of enzyme, purification of amino acids, testing for the dehairing activity, effect of inhibitors, temperature and pH, large-scale production of protease, immobilization of enzyme and application of the resulted enzyme and amino acids in different industries.

References:

Ali, T., Ali, N., and Mohamed L. 2011. Production, purification and some properties of extracellular keratinase from feather -degradation by *Aspergillus oryzae* NRRll-447. Journal of Applied Sciences in Environmental Sanitation. 6(2): 123-136.

Anwar, A. and Saleemuddin, M. 1998. Alkaline proteases. A Review Bioresource Technology. 64: 175 – 183.

Bernhard, K., Schrempf, H., and Goebel, W. 1978. Bacteriocin and antibiotic resistance plasmids in *Bacillus cereus* and *Bacillus subtilis*. J. of Bacteriology. 133(2): 897 – 903.

Cai, C., Chen, G., Jiong–jiong, Qi., Yin, Y. and Zheng, X. 2008. Purification and characterization of keratinase from a new *Bacillus subtilis* strain. Journal of Zhejiang University Science. 9(9): 713–720

El-Shafei, H., Abdel-Aziz, M., Ghaly, M., and Abdalla, A. 2010. Optimizing some factors affecting alkaline protease production by a marine bacterium *Streptomyces albidoflavus*. In: Proceeding of Fifth Scientific Environmental Conference, Zagazig University. 125 – 142

Gradišar, H., Friedrich, J., Križaj, I., and Jerala, R. 2005. Similarities and specificities of fungal keratinolytic proteases: Comparison of keratinases of *Paecilomyces marquandii* and *Doratomyces microsporus* to some known proteases. Appl. Environ. Microbiol. 71(7): 3420–3426.

Han, Y. and Parsons CM. 1991. Protein and amino acid quality of feather meals. Poultry Science. 70: 812 – 822.

Kainoor, S. and Naik G. R. 2010. Production and characterization of feather degrading keratinase from *Bacillus sp.* JP99. Indian Journal of Biotechnology. 9:384-390

Laemli U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680- 685.

Lin, X., Lee, C. G., Casle, E., and Shih, J. C. H. 1992. Purification and characterization of a keratinase from a feather degrading *Bacillus licheniformis* strain. Applied and Environmental Microbiology. 58(10): 3271 – 3275

Lin, X., Shih, J., and Swaisgood, H. 1996. Hydrolysis of feather keratin by immobilized keratinase. Applied and Environmental Microbiology. 62(11): 4273–4275

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randell, R. J. 1951. Protein measurement with the folin phenol reaction. J. Biol. Chem. 193: 265 – 275.

Matsuzawa, H., Tokugawa, K., Hamaoki, M., Mizoguchi, M., Taguchi, H., Terada, I., Kwon, S., and Ohta, T. 1988. Purification and characterization of aqualysin I (a thermophilic alkaline serine protease) produced by *Thermus aquaticus* YT-1. Eur. J. Biochem. 171: 441- 447.

Pearce, K. N., Karahalios, D., and Friedman, M. 1988. Ninhydrin assay for proteolysis in ripening cheese. J. of Food Science. 53(2): 432 – 435.

Pelczar, M. J. and Chan, E.C. 1977. Laboratory Exercises in Microbiology, 4th edition, McGraw Hill, Inc.

Porres, J. M., Benito, M. J., and Lei, X. G. 2002. Functional expression of keratinase (kerA) gene from *Bacillus licheniformis* in *Pichia pastoris*. Biotechnology Letters. 24(8): 631-636.

Riffel, A. and Brandelli, A. 2006. Keratinolytic bacteria isolated from feather waste. Brazilian Journal of Microbiology. 37: 395–399

Savitha, G., Joshi, M., Ejashwini, N., Revati, R., Sridevi, R., and Roma, D. 2007. Isolation, identification and characterization of a feather degrading bacterium. International Journal of Poultry Science. 6(9): 689-693

Sutar, I. I., Srinivasan, M. C., and Vartak H. G. 1991. A low molecular weight alkaline protienase from *Conidiobolus coronatus*. Biotechnology Letters. 13(2): 119 – 124.

Upton, M. and Fogarty, W. M. 1977. Production and purification of thermostable amylase and protease of *Thermomonospora viridis*. Applied and Environmental Microbiology. 33(1): 59-64.

Zaghloul, T. I. Abdel –Aziz, A., and Mostafa, M. H. 1994. High level of expression and stability of the cloned alkaline protease (aprA) gene in *Bacillus subtilis*. Enzyme Microb. Technology. 16: 537.

Zaghloul, T. I. Haroun, M. A. El-Gayar, K., and Abdelal, A. 2004. Recycling of Keratin-containing materials (Chicken Feather) through genetically engineered bacteria. Polymer-Plastics Technology and Engineering. 43(6): 1589 – 1599.

Zaghloul, T. I. 1986. Experssion of TN9 – derived chloramphenicol acetyl transferase gene fusions in *Bacillus subtilis* and *Echerichia coli*. Ph.D. Thesis. University of California, Davis, CA., USA.

Živkovici, L., Cvijovic, G., and Karadzic, I. 2010. Isolation and partial characterization of protease from *Pseudomonas aeruginosa* ATCC 27853. J. Serb. Chem. Soc. 75(8): 1041–1052

تنقية إنزيم البروتييز القاعدي من مخلف ريش الدواجن المتحلل بواسطة سلالة بكتيريا الباسيليس سابتيليس (*B. Subtilis*) المعدلة وراثيا

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الملخص:

ينتج إنزيم البروتييز القاعدي من عملية التحلل الهوائي لمخلفات ريش الدواجن بواسطة بكتيريا الباسيليس المعدلة وراثيا في بيئة تحتوي على 2% من ريش الدواجن لمدة 48 ساعة. وقد كانت درجة الأس الهيدروجيني ودرجة الحرارة المثلى لنشاط هذا الإنزيم هما 7.2 من 45 درجة مئوية على الترتيب. وقد تم ترسيب الإنزيم من محلوله بواسطة كبريتات الأمونيوم (درجة تشبع 65%) متبوعا بعملية الديلزة (الميز الغشائي) عند أس هيدروجيني 7.2. ولقد تم تنقية الإنزيم باستخدام عمود التبادل الأيوني ثم عمود الترشيح سيفادكس جي 50 وتم تتبع مرور الإنزيم بقياس المحتوى البروتيني للراشح من الأعمدة عن طريق قياس درجة الامتصاص الضوئي على طول موجة (280) نانوميتر. تم تنقية الإنزيم بعد عملية الديناليسيس 18.7 مرة وبحصيلة قدرها 91% وبعد التبادل الأيوني 56 مرة وبحصيلة 84.6% وبعد عمود الترشيح 75.5 مرة وبحصيلة 68.5%. باختبار عملية التنقية بالفصل الكهربي ظهر مكون واحد مما يدل على نقاوة الأنزيم بالمقارنة بالعينة قبل التنقية وقدر الوزن الجزيئي بـ 27 كيلو دالتون.

الكلمات المفتاحية: البكتريا المحللة للريش، تنقية البروتييز، الكيراتين.